

OPSONIC AND PROTECTIVE
MONOCLONAL AND CHIMERIC ANTIBODIES
SPECIFIC FOR LIPOTEICHOIC ACID OF GRAM POSITIVE BACTERIA

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CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of Provisional Patent Application Serial No. 60/049,871, filed June 16, 1997, which application is specifically incorporated herein by reference.

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FIELD OF THE INVENTION

This invention in the fields of immunology and infectious diseases relates to antibodies that are specific for Gram positive bacteria, particularly to lipoteichoic acids exposed on the surface of the bacteria. The invention includes monoclonal and chimeric antibodies, as well as fragments, regions and derivatives thereof. This invention also relates to the epitope to which the antibodies of the invention bind as well as the sequences, fragments, and regions of the epitopes. Both the antibodies and peptides that encompass the epitope, and regions and fragments thereof, may be used for diagnostic, prophylactic and therapeutic applications.

BACKGROUND OF THE INVENTION

Man has long battled bacterial infections, and no one can doubt the tremendous successes obtained. Before the discovery and development of antibiotics, death due to a bacterial infection was frequently rapid and inevitable. Surgical procedures and sanitary conditions have vastly improved from the time when amputation was associated with a 50 percent mortality rate.

Nonetheless, the battle has not been won. Undoubtedly a significant part of the problem is that bacteria are the product of nearly 3 billion years of natural selection from which they have emerged as an immensely diverse group of organisms that colonize almost all parts of the world and its inhabitants. To begin to understand bacteria requires categorization, and the most fundamental categories for bacteria are their response to the Gram stain, yielding (for the most part) Gram positive bacteria and Gram negative bacteria.

The difference in response to the Gram stain results from differences in bacterial cell walls. The cells walls of Gram negative bacteria are made up of a unique outer membrane of two opposing phospholipid-protein leaflets, with an ordinary phospholipid in the inner leaflet but the extremely toxic lipopolysaccharide in the outer leaflet. The cell walls of Gram positive bacteria seem much simpler in comparison, containing two major components, peptidoglycan and teichoic acids plus additional carbohydrates and proteins depending on the species.

Of the Gram positive bacteria, one of the most common genera is *Staphylococcus*. Staphylococci commonly colonize humans and animals and are an important cause of human morbidity and mortality, particularly in hospitalized patients. Staphylococci are prevalent on the skin and mucosal linings and, accordingly, are ideally situated to produce both localized and systemic infections.

There are two main groups of Staphylococci divided according to the production of "coagulase," an enzyme that causes fibrin to coagulate and to form a clot: coagulase positive and coagulase negative. The coagulase positive *Staphylococcus* species most

frequently pathogenic in humans is *Staphylococcus aureus*. *S. aureus* is the most virulent *Staphylococcus* and produces severe and often fatal disease in both normal and immunocompromised hosts. *Staphylococcus epidermidis* is the most common coagulase negative species.

In recent years, *S. epidermidis* has become a major cause of nosocomial infection in patients whose treatments include the placement of foreign objects such as cerebrospinal fluid shunts, cardiac valves, vascular catheters, joint prostheses, and other implants into the body. *S. epidermidis* and *S. aureus* are common causes of post-operative wound infections and *S. epidermidis* has also become a common cause of peritonitis in patients with continuous ambulatory peritoneal dialysis. In a similar manner, patients with impaired immunity and those receiving parenteral nutrition through central venous catheters are at high risk for developing *S. epidermidis* sepsis. (C.C. Patrick, J. Pediatr., 116:497 (1990)). *S. epidermidis* is now recognized as a common cause of neonatal nosocomial sepsis. Infections frequently occur in premature infants that have received parenteral nutrition which can be a direct or indirect source of contamination.

Staphylococcal infections are difficult to treat for a variety of reasons. Resistance to antibiotics is common and becoming more so. See L. Garrett, The Coming Plague, "The Revenge of the Germs or Just Keep Inventing New Drugs" Ch. 13, pgs. 411-456, Farrar, Straus and Giroux, NY, Eds. (1994). In one study, the majority of Staphylococci isolated from blood cultures of septic infants were multiply resistant to antibiotics (A. Fleer et al., Pediatr. Infect. Dis. 2:426 (1983)). A more recent

homologous strain but not to heterologous strains. Accordingly, based on the studies of Fattom et al., and Timmerman et al., and others in the field (and in contrast to our own studies), one would not expect that an antibody that is broadly reactive to multiple strains of *S. epidermidis* and to *S. aureus* would have opsonic activity against both. This is particularly true for antibodies that bind to both coagulase positive and coagulase negative Staphylococci.

Accordingly, there is a need in the art to provide monoclonal antibodies that can bind to *Staphylococcus* of both coagulase types and that can enhance phagocytosis and killing of the bacteria and thereby enhance protection *in vivo*. There is also a need in the art for the epitope of the site to which such antibodies can bind so that other antibodies with similar abilities can be identified and isolated.

There is a related need in the art for humanized or other chimeric human/mouse monoclonal antibodies. In recent well publicized studies, patients administered murine anti-TNF (tumor necrosis factor) monoclonal antibodies developed anti-murine antibody responses to the administered antibody. (Exley A.R., et al., Lancet 335:1275-1277 (1990)). This type of immune response to the treatment regimen, commonly referred to as the HAMA response, decreases the effectiveness of the treatment and may even render the treatment completely ineffective. Humanized or chimeric human/mouse monoclonal antibodies have been shown to significantly decrease the HAMA response and to increase the therapeutic effectiveness. See LoBuglio et al., P.N.A.S. 86:4220-4224 (June 1989).

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a schematic representation of lipoteichoic acid (LTA) in the Gram positive bacterial cell wall.

Figure 2 depicts antibody regions, such as the heavy chain constant region (C_H), the heavy chain variable region (V_H), the light chain constant region (C_L), and the light chain variable region (V_L).

Figure 3 demonstrates the enhancement of survival after administration of MAB 96-110 in a lethal neonatal model of coagulase positive staphylococcus sepsis.

Figure 4 demonstrates enhancement of survival in adult mice infected with coagulase negative staphylococci after administration of MAB 96-110. Approximately 23 hours after infection, 70% of the animals treated with MAB 96-110 were alive compared with 20% of animals not given antibody.

Figure 5 (SEQ ID NOS 4 & 5, and 6 & 7, respectively) provides a list of 18 resulting sequences for the 6mer library panning.

Figure 6 (SEQ ID NOS 8-43, respectively) provides a list of the 18 resulting sequences for the second experiment 15mer library panning.

Figure 7 (SEQ ID NOS 44 & 45, and 46 & 47, and 48 & 49, respectively) provides a list of the 17 resulting sequences for the first experiment 15mer library panning.

Figure 8 (SEQ ID NOS 50-67, respectively) provides a master list compiled from the common resulting peptide sequences from all the pannings.

Figure 9 sets forth a comparison of the optical density signals of each phage isolate at 6.25×10^{11} vir/ml.

Figure 10 shows the strategy for cloning the variable region gene fragments.

Figure 11 (SEQ ID NOS 68-85, respectively) lists the oligonucleotides primers used.

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A2 } Figure 12 (SEQ ID NOS 86-105, respectively) provides the final consensus DNA sequence of the heavy and light chain variable regions.

Figure 13 demonstrates the re-amplification of the variable region gene fragments.

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Figure 14 sets forth the plasmid map for pJRS334.

Figure 15 provides the results of the antibody production assay, showing that the transfection of cells with the plasmid construct results in the production of a molecule containing both human IgG and kappa domains.

Figure 16 provides the results of the activity assay, demonstrating that the transfection of cells with the plasmid construct results in the production of a molecule that binds to the Hay antigen.

Figure 17 depicts the opsonic activity of the chimeric monoclonal antibody 96-110 for *S. epidermidis* in a neutrophil mediated opsonophagocytic bactericidal assay.

Figure 18 demonstrates the enhancement of survival after administration of MAB 96-110 in a lethal model of *S. epidermidis* sepsis.

Figure 19 depicts the effect of the chimeric monoclonal antibody 96-110 on the survival of adult mice after intraperitoneal challenge with *S. epidermidis*.

Figure 20 sets forth the effect of the chimeric monoclonal antibody 96-110 on bacteremia in a lethal *S. epidermidis* model.

Figure 21 depicts bacteremia levels four hours after infection with *S. epidermidis* at different doses of the chimeric monoclonal antibody 96-110.

Figure 22 sets forth the effect of the chimeric monoclonal antibody 96-110 on survival in a lethal neonatal *S. epidermidis* sepsis model.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides anti-lipoteichoic acid (LTA) murine antibodies (including monoclonal antibodies) and chimeric murine-human antibodies, and fragments, derivatives, and regions thereof, which bind to and opsonize whole Gram positive cocci such as *Staphylococcus* to thereby enhance phagocytosis and killing of such bacteria *in vitro* and which enhance protection from lethal infection of such bacteria *in vivo*. The antibodies, fragments, regions, and derivatives thereof of the invention preferably recognize and bind to an epitope of LTA that can block the binding of Gram positive bacteria to epithelial cells, such as human epithelial cells. Accordingly, the invention provides broadly reactive and opsonic antibodies for the diagnosis, prevention, and/or treatment of bacterial infections caused by Gram positive bacteria.

The antibodies of the invention are broadly reactive with Gram positive bacteria, meaning that they selectively recognize and bind to Gram positive bacteria and do not recognize or bind to Gram negative bacteria. Any conventional binding assay can be used to assess this binding, including for example, the enzyme linked immunosorbent assay described below. The basis of the binding is the presence of LTA exposed on the surface of the cell wall of Gram positive bacteria.

As noted above, the cell walls of Gram positive bacteria characteristically contain peptidoglycans such as murein as well as teichoic acids. Teichoic acids are polymers of either glycerol phosphate or ribitol phosphate with various sugars, amino sugars, and amino acids as substituents. Although the lengths of the chains and the nature and location of the substituents vary from species to species and sometimes between

species, in general teichoic acids make up a major part of the cell wall. The teichoic acids related to this invention are lipoteichoic acids which are teichoic acids made up of glycerol phosphate which is primarily linked to a glycolipid in the underlying cell membrane. Although the precise structure of LTA in the Gram positive bacterial cell wall is not known, a standard schematic representation commonly accepted in the art is set forth in Figure 1. Accordingly, the antibodies of the claimed invention are broadly reactive because they recognize and bind to the lipoteichoic acids that are characteristically surface exposed on Gram positive bacteria.

The antibodies of the invention are also opsonic, or exhibit opsonic activity, for Gram positive bacteria. As those in the art recognize, "opsonic activity" refers to the ability of an opsonin (generally either an antibody or the serum factor C3b) to bind to an antigen to promote attachment of the antigen to the phagocyte and thereby enhance phagocytosis. Certain bacteria, especially encapsulated bacteria which resist phagocytosis due to the presence of the capsule, become extremely attractive to phagocytes such as neutrophils and macrophages when coated with an opsonic antibody and their rate of clearance from the bloodstream is strikingly enhanced. Opsonic activity may be measured in any conventional manner as described below.

The ability of the anti-LTA antibodies of the invention to bind to and opsonize Gram positive bacteria and thereby enhance phagocytosis and cell killing *in vitro* and to enhance protection *in vivo* is completely unexpected because anti-LTA antibodies have been reported to lack opsonic activity. Indeed, anti-LTA antibodies have been often used as controls.

For example, Fattom et al., J. Clin. Micro. 30(12):3270-3273 (1992) examined the opsonic activity of antibodies induced against type specific capsular polysaccharide of *S. epidermidis*, using as controls antibodies induced against teichoic acids and against *S. hominus*. While type-specific antibodies were highly opsonic, anti-techoic acid antibodies were not different from the anti-*S. hominus* antibodies.

Similarly, in Kojima et al., J. Infect. Dis. 162:435-441 (1990), the authors assessed the protective effects of antibody to capsular polysaccharide/adhesion against catheter-related bacteremia due to coagulase negative Staphylococci and specifically used a strain of *S. epidermidis* that expresses teichoic acid as a control. See page 436, Materials and Methods, left column, first ¶; right column, third ¶. In a later study, the authors reached a more explicit conclusion against the utility of anti-techoic antibodies:

Immunization protocols designed to elicit antibody to techoic acid but not to PS/A afforded no protection against bacteremia or endocarditis.

Takeda, et al., Circulation 86(6):2539-2546 (1991).

Contrary to the prevailing view in the field, the broadly reactive opsonic antibodies against the LTA of Gram positive bacteria, including *S. aureus* and *S. epidermidis*, of the invention satisfy a clear need in the art. As described in the background section, both *S. aureus* and *S. epidermidis* are common causes of post-operative wound infections; *S. epidermidis* has become a major cause of nosocomial infections in patients whose treatments include the placement of foreign objects; *S. epidermidis* has become a common cause of peritonitis in patients with continuous

ambulatory peritoneal dialysis; and *S. epidermidis* is now recognized as a common cause of neonatal sepsis.

Indeed, our laboratory has recently focused tremendous efforts to find broadly opsonic antibodies as detailed in a recent series of four related applications and one issued patent, specifically:

USSN 08/296,133, filed August 26, 1994, of Gerald W. Fischer, entitled "Directed Human Immune Globulin for the Prevention of Staphylococcal Infections;"

U.S. Patent No. 5,571,511, issued November 5, 1996 to Gerald W. Fischer, entitled "Broadly Reactive Opsonic Antibodies that React with Common Staphylococcal Antigens;"

USSN 08/466,059, filed June 6, 1995, of Gerald W. Fischer, entitled "*In Vitro* Methods for Identifying Pathogenic Staphylococcus, For Identifying Immunoglobulin Useful for the Treatment of Pathogenic Staphylococcus Infections, and *In Vitro* Methods Employing such Immunoglobulins;" and

USSN 08/308,495, filed September 21, 1994, of to Gerald W. Fischer, entitled "Broadly Reactive Opsonic Antibodies that React with Common Staphylococcal Antigens,"

all of which are specifically incorporated herein by reference.

This series of applications and the issued patent describe the search for broadly reactive opsonic antibodies particularly against Staphylococci. In rough chronological order, the "Directed Human Immune Globulin" application describes the selection and use of Directed Human Immune Globulin to prevent or treat infections caused by *S. epidermidis* which contains antibodies with the ability to bind to surface antigens of *S. epidermidis* in an ELISA and the exhibition of greater than 80% opsonophagocytic bactericidal activity against *S. epidermidis* in a particularly described *in vitro* assay. The

issued patent claims describes for the first time a particular strain of *S. epidermidis* that identifies broadly reactive opsonic antibodies against both coagulase positive and coagulase negative Staphylococci and specifically claims an antigen preparation isolated from *S. epidermidis* strain Hay ATCC 55133, deposited on December 19, 1990, which generates broadly reactive opsonic antibody which specifically reacts in an assay with *S. epidermidis* serotypes I, II and III, and which exhibits opsonic activity greater than 70%. The "In Vitro Methods" application describes the use of a Serotype II *S. epidermidis*, such as the Hay strain, that identifies pathogenic Staphylococcus infections. The fourth application in the chain describes a surface protein identified on the Hay strain that can induce broadly reactive opsonic antibodies.

Nonetheless, the search continued for antibodies, both polyclonal and monoclonal, that are broadly reactive and opsonic against all Gram positive bacteria and has culminated in the present invention. Having discovered the Hay strain and determined its unique ability to generate broadly opsonic antibodies against Staphylococci, it was used as the basis for this search.

As set forth in Example 1, mice were immunized with whole strain Hay *S. epidermidis* from which hybridomas were produced. In screening the hybridomas for antibodies, the antibodies of one clone (first designated 96-105CE11 IF6 and later designated 96-110 MAB) exhibited a strong IgG reaction (Tables 1 and 2) and, in further tests, was found to bind very strongly to Gram positive bacteria such as to strain Hay, to all three serotypes of *S. epidermidis*, to *S. hemolyticus*, *S. hominus*, and two

serotypes of *S. aureus* (Tables 3-6) but not to the Gram negative control, *Haemophilus influenza*.

Similar to the antibodies described in the Fischer applications and patent set forth above, the antibody of the present invention exhibits very strong binding, i.e., O.D.s of around twice background in an enzyme-linked immunosorbent assay (described below), against strain Hay. In a preferred embodiment, the level of high binding is equal to or greater than five times background. In other embodiments, the level of high binding is equal to or greater than 10 times background. Of course, any meaningful increase over background (the level observed when all the reagents other than the antibody being tested) will be recognized by skilled persons in the art as high binding and therefor within the scope of the invention.

Also as described in the Fischer applications and patent, high binding has been found to correlate with opsonic activity. As set forth in Example 2, in a neutrophil mediated bactericidal assay (described below), the 96-110 MAB exhibited enhanced opsonization against the prototypic coagulase negative bacteria, *S. epidermidis*, and against the prototypic coagulase positive bacteria, *S. aureus*. With this level of opsonic activity, an antibody should enhance phagocytosis and cell killing of both coagulase negative and coagulase positive bacteria.

The term "enhanced" refers to activity that measurably exceeds background at a valuable level. The level deemed valuable may well vary depending on the specific circumstances of the infection, including the type of bacteria and the severity of the infection. For example, for enhanced opsonic or phagocytic activity, in a preferred

embodiment, an enhanced response is equal to or greater than 75% over background. In another preferred embodiment, the enhanced response is equal to or greater than 80% over background. In yet another embodiment, the enhanced response is equal to or greater than 90% over background.

To confirm that the antibody, previously shown to be broadly reactive as well as opsonic, would be protective *in vivo*, MAB 96-110 was assessed in a lethal infection model in both neonatal rats and adult mice. As set forth in Example 3, survival in control animals given either no therapy, saline, or control MAB, ranged from 0 to less than 10%. However, MAB 96-110 enhanced the survival to 50% or greater.

Where, as here, the enhancement measured is of survival, the preferred increase over background may be more modest than above. Thus, an increase in survival of 25% may be an enhanced response. In other embodiments, enhanced survival may be greater than 50%. Again, the person of skill in the art would recognize other meaningful increases in survival as within the invention.

In view of the impressive opsonic activity *in vitro* as well as the protective activity *in vivo* of MAB 96-110, we sought the identity of the epitope of the antigen to which it bound. An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody and which is also capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one or more epitopes. An "epitope" analogously means that portion of the molecule that is capable of being recognized by and bound by an antibody. In general, epitopes consist of chemically active surface groupings of molecules such as amino acids or sugar side

chains that have specified three dimensional structural and specific charge characteristics.

In a series of panning experiments set forth in Examples 4-6, we identified peptide sequences to which MAB 96-110 bound strongly. These sequences provide at least peptide mimics of the epitope to which MAB 96-110 bound. Thus, one aspect of the present invention involves a peptide having the sequence

W R M Y F S H R H A H L R S P (SEQ ID NO 1)

and another aspect of the invention involves a peptide having the sequence

W H W R H R I P L Q L A A G R (SEQ ID NO 2).

Of course, the epitope of the invention may be identical to one of these sequences or may be substantially homologous to these sequences such that the anti-LTA antibodies of the invention will bind to them. Alternatively, the substantially homologous sequences of the invention are those that are able to induce the anti-LTA antibodies of the invention. Other peptide epitope mimics within the invention may vary in length and sequence from these two peptides.

The present invention also encompasses recombinant epitopes, epitope mimics, and antigens. The DNA sequence of the gene coding for the isolated antigen can be identified, isolated, cloned, and transferred to a prokaryotic or eukaryotic cell for expression by procedures well-known in the art. For example, procedures are generally described in Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Springs Harbor Press, Cold Spring Harbor, New York (1989) incorporated by reference.

To confirm the specificity of the peptides for the monoclonal antibody, it was tested in a competitive inhibition assay and found to inhibit binding of MAB 96-110 to strain Hay. See Example 6.

To determine the protein of which such sequences are a part, we compared the peptide sequences to the sequences of proteins but, as set forth in Example 7, failed to identify any known protein. Accordingly, we expanded our search of other antigen candidates. Because the peptide sequence was small and had successfully inhibited the binding of MAB 96-110 to strain Hay and because MAB 96-110 bound to and opsonized all three serotypes of *S. epidermidis* as well as to both coagulase negative and coagulase positive bacteria, we assessed the possibility that the peptide was part of a surface exposed lipoteichoic acid. To our surprise, as set forth in Example 7, we found that MAB 96-110 bound to the LTAs of several Gram positive bacteria such as *S. mutans*, *S. aureus*, *S. faecalis*, *S. pyogenes* (group A Streptococcus).

Thus, the present invention includes antibodies that are capable of binding to the LTA of Gram positive bacteria, including both coagulase negative and coagulase positive bacteria, and of enhancing the opsonization of such bacteria. These anti-LTA antibodies include polyclonal antibodies as well as monoclonal antibodies produced by the hybridomas of the invention, such as MAB 96-110 as well as other monoclonal antibodies, fragments and regions thereof, as well as derivatives thereof. As set forth above, the strength of the binding may range from twice above background, to five- and ten-times above background. In addition, the antibodies, fragments, regions, and

derivatives of the present invention are capable of enhancing the opsonization of such bacteria, at rates ranging from 75% and up.

The "fragments" of the antibodies of the invention include, for example, Fab, Fab', F(ab')₂, and SFv. These fragments are produced from intact antibodies using methods well known in the art such as, for example, proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂). The "regions" of the antibodies of the present invention include a heavy chain constant region (H_c or C_H), a heavy chain variable region (H_v or V_H), a light chain constant region (L_c or C_L), and a light chain variable region (L_v or V_L) (Figure 2). The light chains may be either a lambda or a kappa chain.

In a preferred aspect of the invention, the regions include at least one heavy chain variable region or a light chain variable region which binds a portion of LTA, including for example the specific antigen binding sites (that which binds to the epitope) of the two regions. In another embodiment, these two variable regions can be linked together as a single chain antibody. While a full length heavy chain may be critical for opsonic activity and enhance anti-cytokine (anti-inflammatory) activity, the antibody fragments encompassing the variable regions may be suitable for inhibition of bacterial binding to epithelial cells and may also be anti-inflammatory.

In a particularly preferred aspect of the invention, the antibody is a chimeric mouse/human antibody made up of regions from the anti-LTA antibodies of the invention together with regions of human antibodies. For example, a chimeric H chain can comprise the antigen binding region of the heavy chain variable region of the anti-

LTA antibody of the invention linked to at least a portion of a human heavy chain constant region. This humanized or chimeric heavy chain may be combined with a chimeric L chain that comprises the antigen binding region of the light chain variable region of the anti-LTA antibody linked to at least a portion of the human light chain constant region.

The chimeric antibodies of the invention may be monovalent, divalent, or polyvalent immunoglobulins. For example, a monovalent chimeric antibody is a dimer (HL) formed by a chimeric H chain associated through disulfide bridges with a chimeric L chain, as noted above. A divalent chimeric antibody is a tetramer ($H_2 L_2$) formed by two HL dimers associated through at least one disulfide bridge. A polyvalent chimeric antibody is based on an aggregation of chains.

A particularly preferred chimeric antibody of the invention is described in Examples 8-10 which set forth in detail the preparation of a preferred chimeric IgG antibody (and in Examples 11-13 which describe the functional activity of this preferred chimeric antibody). Of course, other chimeric antibodies composed of different sections of the anti-LTA antibodies of the invention are within the invention. In particular, the heavy chain constant region can be an IgM or IgA antibody.

In addition to the protein fragments and regions of the antibodies, the present invention also encompasses the DNA sequence of the gene coding for the antibodies as well as the peptides encoded by the DNA. Particularly preferred DNA and peptide sequences are set forth in Figure 12. That figure provides the variable regions of both the heavy and light chains of MAB 96-110, including the Complementarity Determining

Regions ("CDR"), the hypervariable amino acid sequences within antibody variable regions which interact with amino acids on the complementary antigen. The invention includes these DNA and peptide sequences as well as DNA and peptide sequences that are homologous to these sequences. In a preferred embodiment, these sequences are 70 % homologous although other preferred embodiments include sequences that are 75%, 80%, 85%, 90%, and 95% homologous. Determining these levels of homology for both the DNA and peptide sequence is well within the routine skill of those in the art.

The DNA sequences of the invention can be identified, isolated, cloned, and transferred to a prokaryotic or eukaryotic cell for expression by procedures well-known in the art. Such procedures are generally described in Sambrook et al., *supra*, as well as Current Protocols in Molecular Biology (Ausubel et al., eds., John Wiley & Sons, 1989), incorporated by reference. In one preferred embodiment, the CDR can be grafted onto any human antibody frame using techniques standard in the art, in such a manner that the CDR maintains the same binding specificity as in the intact antibody.

In addition, the DNA and peptide sequences of the antibodies of the invention, including both monoclonal and chimeric antibodies, may form the basis of antibody "derivatives," which include, for example, the proteins or peptides encoded by truncated or modified genes. Such proteins or peptides may function similarly to the antibodies of the invention. Other modifications, such as the addition of other sequences that may enhance the effector function, which includes phagocytosis and/or killing of the bacteria, are also within the present invention.

A therapeutically effective amount is an amount reasonably believed to provide some measure of relief or assistance in the treatment of the infection. Such therapy as above or as described below may be primary or supplemental to additional treatment, such as antibiotic therapy, for a staphylococcal infection, an infection caused by a different agent, or an unrelated disease. Indeed, combination therapy with other antibodies is expressly contemplated within the invention.

A further embodiment of the present invention is a method of preventing such infections, comprising administering a prophylactically effective amount of a pharmaceutical composition comprising the anti-LTA antibody (whether polyclonal or monoclonal or chimeric, including fragments, regions, and derivatives thereof) and a pharmaceutically acceptable carrier.

A prophylactically effective amount is an amount reasonably believed to provide some measure of prevention of infection by Gram positive bacteria. Such therapy as above or as described below may be primary or supplemental to additional treatment, such as antibiotic therapy, for a staphylococcal infection, an infection caused by a different agent, or an unrelated disease. Indeed, combination therapy with other antibodies is expressly contemplated within the invention.

In another embodiment, the peptide which mimics the LTA epitope would be useful to prevent binding of Gram positive bacteria to epithelial cells and thereby inhibit colonization. For example, a pharmaceutical composition containing such a peptide may be administered intranasally to prevent an infection or to minimize a current infection.

Yet another preferred embodiment of the present invention is a vaccine comprising the epitope, epitope mimic, or other part of the LTA antigen and a pharmaceutically acceptable carrier. Upon introduction into a host, the vaccine generates an antibody broadly protective and opsonic against infection by Gram positive bacteria. The vaccine may include the epitope, an epitope mimic, any mixture of epitopes and epitope mimics, the antigen, different antigens, or any combination of epitopes, epitope mimics and antigens.

Vaccinations are particularly beneficial for individuals known to be or suspected of being at risk of infection by Gram positive bacteria. This includes patients receiving body implants, such as valves, patients with indwelling catheters, patients preparing to undergo surgery involving breakage or damage of skin or mucosal tissue, certain health care workers, and patients expected to develop impaired immune systems from some form of therapy, such as chemotherapy or radiation therapy.

Treatment comprises administering the pharmaceutical composition (including antibodies and vaccines) by intravenous, intraperitoneal, intracorporeal injection, intra-articular, intraventricular, intrathecal, intramuscular, subcutaneous, intranasally, intravaginally, orally, or by any other effective method of administration. The composition may also be given locally, such as by injection to the particular area infected, either intramuscularly or subcutaneously. Administration can comprise administering the pharmaceutical composition by swabbing, immersing, soaking, or wiping directly to a patient. The treatment can also be applied to objects to be placed within a patient, such as dwelling catheters, cardiac valves, cerebrospinal fluid shunts,

joint prostheses, other implants into the body, or any other objects, instruments, or appliances at risk of becoming infected with a Gram positive bacteria, or at risk of introducing such an infection into a patient.

As a particularly valuable corollary of treatment with the compositions of the invention (including all anti-LTA antibodies (whether polyclonal or monoclonal or chimeric, including fragments, regions, and derivatives thereof), all pharmaceutical compositions based on such antibodies, as well as on epitope, epitope mimics, or other part of the LTA antigen and vaccines based on such epitope or antigens) is the reduction in cytokine release that results from the introduction of the LTA of a Gram positive bacteria. As is now recognized in the art, LTA induces cytokines, including for example tumor necrosis factor alpha, Interleukin 6, and interferon gamma. See Takada et al., Infection and Immunity, 63 (1):57-65 (January 1995). Accordingly, the compositions of the invention may enhance protection at three levels: (1) by binding to LTA on the bacteria and thereby blocking the initial binding to epithelial cells and preventing subsequent invasion of the bacteria; (2) by enhancing opsonization of the bacteria and thereby enhancing clearance of the bacteria from tissues and blood; and/or (3) by binding to LTA and partially or fully blocking cytokine release and modulating the inflammatory responses to prevent shock and tissue destruction.

Having generally described the invention, it is clear that the invention overcomes some of the potentially serious problems described in the Background regarding the development of antibiotic resistant Gram positive bacteria. As set forth above, Staphylococci and streptococci (such as *S. faecalis*) have become increasingly

resistant and, with the recent spread of vancomycin resistant strains, antibiotic therapy may become totally ineffective.

Particular aspects of the invention are now presented in the form of the following "Materials and Methods" as well as the specific Examples. Of course, these are included only for purposes of illustration and are not intended to be limiting of the present invention.

MATERIALS AND METHODS

Bacteria

S. epidermidis, strain Hay, was deposited at the ATCC on December 19, 1990 under Accession No. 55133.

Hybridoma

Hybridoma 96-110 was deposited at the ATCC on June 13, 1997 under Accession No. HB-12368.

Isotype Determination Assay

Isotype was determined using an isotype kit obtained from Zymed Laboratories. The kit can be ordered under number 90-6550.

Binding Assays

In the binding assay of the invention, immunoglobulin is reacted with a preparation of a Staphylococcal organism. The binding assay is preferably an enzyme-linked immunosorbent assay (ELISA) or a radioimmunoassay (RIA), but may also be an agglutination assay, a coagglutination assay, a colorimetric assay, a

fluorescent binding assay, or any other suitable binding assay that is known in the art. The assay can be performed by competitive or noncompetitive procedures with results determined directly or indirectly.

The Staphylococcus preparation may be fixed to a suitable solid support, such as a glass or plastic plate, well, bead, micro-bead, paddle, propeller, or stick. The solid support is preferably a titration plate. The fixed preparation is incubated with immunoglobulin, which is isolated or in a biological fluid such as ascites, and the amount of binding determined. A positive reaction occurs when the amount of binding observed for the test sample is greater than the amount of binding for a negative control. A negative control is any sample known not to contain antigen-specific immunoglobulin. Positive binding may be determined from a simple positive/negative reaction or from the calculation of a series of reactions. This series may include samples containing measured amounts of immunoglobulin that specifically bind to the fixed antigen, creating a standard curve from which the amount of antigen-specific immunoglobulin in an unknown sample can be determined. Alternatively, antibody can be fixed to a solid support and immunoglobulin identified by its ability to bind a bacterial preparation bound to the fixed antibodies.

The specific of the assays used in the Examples are set forth below:

Immunoassay on Methanol-Fixed Bacterial: Heat-killed bacteria were suspended in normal saline at an $OD_{650}=0.600$. Bacteria in 5 mls of the suspension were pelleted by centrifugation (approximately 1800 X g, 15 minutes, 10-15°C). The supernatant was discarded and the pellet resuspended into 12 mls of methanol

(MeOH). One hundred microliters of the suspension in MeOH was distributed into each well of Nunc Maxisorp Stripwells. The MeOH was allowed to evaporate, fixing the bacteria to the plastic. The bacteria-coated stripwells were stored in plastic bags and used within 2 months of preparation.

For evaluation of antibodies, the bacteria-coated plates were washed once with PBS and non-specific reactive sites on the bacteria were blocked by the addition of 120 ul/well of a solution of 1% bovine serum albumin (BSA) in PBS. After a 30-60 minute incubation, the wells were washed four times with PBS containing 0.05% Tween-20 (PBS-T). Antibody, diluted in PBS-T, was then added to the wells. Supernatants were tested at a dilution of 1:2. Ascites and purified antibody were tested at dilutions indicated in the Tables. After addition of the antibody, the wells were incubated at room temperature for 30-60 minutes in a draft-free environment. The wells were again washed four times with PBS-T and each well received 95 ul of detection antibody: rabbit anti-mouse IgG, conjugated to horse radish peroxidase (HRP) and diluted 1:4000 in PBS-T. The detection antibodies were specific for mouse gamma, mu or alpha heavy chains (Zymed catalog numbers 61-6020, 61-6820 or 61-6720 respectively), as indicated in the Tables.

Following another 30-60 incubation at room temperature, the wells were washed four times with PBS-T and each well received 100 ul of one-component TMB substrate solution (Kirkegaard and Perry Labs catalog number 50-76-05). The wells were incubated in the dark at room temperature for 15 minutes. The reaction was stopped by the addition of 80 ul of TMB stop solution (Kirkegaard and Perry Labs catalog

number 50-85-05) and the absorbance of each well at 450 nm was determined using a Molecular Devices Vmax plate reader.

Immunoassay on LTA's: Reactivity of MAB 96-110 was measured by immunoassay on wells coated with LTA's. LTA's were obtained from Sigma Chemical Company and diluted in PBS to 1 ug/ml. One hundred microliters of the 1 ug/ml solution was distributed into replicate Nunc Maxisorp Stripwells. The LTA was incubated in the wells overnight at room temperature. The unbound material was removed from the wells with four washes of PBS-T. The wells were not blocked with BSA or other proteins. Antibody, diluted in PBS-T, was then added to the wells and the assay continued as described above.

Competitive Inhibition of Antibody of LTA: In order to determine the ability of LTA to inhibit binding of MAB 96-110 to wells coated with MeOH-fixed Strain Hay, a competitive inhibition assay was performed. Wells were coated in MeOH with Strain Hay and blocked with BSA as described above. Fifty ul of LTA from *S. mutans*, *S. aureus* or *S. faecalis* were added to duplicate wells. Six different concentrations of each LTA were tested (from 0.04 to 9.0 ug/ml). LTA's were diluted in PBS-T to obtain the desired concentrations. Immediately after addition of the LTA, 50 ul of purified MAB 96-110 at 1 ug/ml was added to each well. The final dilution of the MAB 96-110 was therefore 0.5 ug/ml. Uninhibited control wells received only PBS-T and MAB without LTA.

Binding of MAB 96-110 to the LTA in the PBS-T solution resulted in a complex of MAB/LTA which was removed from the plate during the subsequent washing step. The

interaction of the MAB 96-110 with the LTA inhibited the antibody from binding to the LTA on the surface of the bacteria and thus reduced the number of MAB 96-110 molecules bound to the MeOH-fixed strain Hay used to coat the wells. Because the number of MAB 96-110 molecules bound to the MeOH-fixed Strain Hay was reduced, the level of binding of the detection antibody (rabbit anti-mouse IgG-HRP) was therefore also decreased, leading to a reduction of color development when compared to wells in which no LTA was present.

Immunoassay with Protein A Method: In order to evaluate monoclonal antibody 96-110 for reactivity with *S. aureus* 5 and *S. aureus* 8, it was necessary to modify the immunoassay procedure described above. Both *S. aureus* strains express Protein A on their surfaces. Because Protein A binds strongly to the constant region of the heavy chains of gamma-globulins, it was possible that false positive results would be obtained due to non-specific binding of the 96-110 antibody to the Protein A molecule. In order to overcome this difficulty, the immunoassay wells were coated with bacteria as described above. However, prior to the addition of the 96-110 antibody to the bacteria-coated wells, the purified monoclonal antibody (MAb) was reacted with a solution of recombinant Protein A conjugated to HRP and diluted 1:500 in PBS-T. This reaction was allowed to proceed for 30 minutes. The wells were washed four times with PBS-T and 100 ul of the solution of Protein A-HRP-MAb was added to the wells. The presence of the Protein A-HRP from the pretreatment prevented the MAb from binding to the Protein A on the *S. aureus* 5 and 8. Furthermore, the binding of the Protein A-HRP to the constant region of the heavy chain did not interfere with the antibody

binding site on the MAb, thereby allowing evaluation of the MAb on *S. aureus* and other bacteria.

The Protein A-HRP-MAb was allowed to react in the coated wells for 30-60 minutes at room temperature. The wells were then washed with PBS-T and TMB substrate solution was added and the assay completed as described above.

Opsonization assays

An opsonization assay can be a colorimetric assay, a chemiluminescent assay, a fluorescent or radiolabel uptake assay, a cell-mediated bactericidal assay, or any other appropriate assay known in the art which measures the opsonic potential of a substance and identifies broadly reactive immunoglobulin. In an opsonization assay, the following are incubated together: an infectious agent, a eukaryotic cell, and the opsonizing substance to be tested, or an opsonizing substance plus a purported opsonizing enhancing substance. Preferably, the opsonization assay is a cell-mediated bactericidal assay. In this *in vitro* assay, the following are incubated together: an infectious agent, typically a bacterium, a phagocytic cell, and an opsonizing substance, such as immunoglobulin. Although any eukaryotic cell with phagocytic or binding ability may be used in a cell-mediated bactericidal assay, a macrophage, a monocyte, a neutrophil, or any combination of these cells, is preferred. Complement proteins may be included to promote opsonization by both the classical and alternate pathways.

The opsonic ability of immunoglobulin is determined from the amount or number of infectious agents remaining after incubation. In a cell-mediated bactericidal assay, this is accomplished by comparing the number of surviving bacteria between two similar

assays, only one of which contains the purported opsonizing immunoglobulin. Alternatively, the opsonic ability is determined by measuring the numbers of viable organisms before and after incubation. A reduced number of bacteria after incubation in the presence of immunoglobulin indicates a positive opsonizing ability. In the cell-mediated bactericidal assay, positive opsonization is determined by culturing the incubation mixture under appropriate bacterial growth conditions. Any significant reduction in the number of viable bacteria comparing pre- and post-incubation samples, or between samples which contain immunoglobulin and those that do not, is a positive reaction.

Clearance/protective assays

Another preferred method of identifying agents for the treatment or prevention of a infection by Gram positive bacteria employs lethal models of sepsis that measure clearance and protection. Such agents can be immunoglobulin or other antimicrobial substances.

A particularly useful animal model comprises administering an antibody and a Gram positive organism to an immunocompromised (e.g., an immature) animal, followed by evaluating whether the antibody reduces mortality of the animal or enhances clearance of the organism from the animal. This assay may use any immature animal, including the rabbit, the guinea pig, the mouse, the rat, or any other suitable laboratory animal. The suckling rat lethal animal model is most preferred. Such a model can readily incorporate an infected foreign body, such as an infected

catheter, to more closely mimic the clinical setting. An alternative model utilizes adult susceptible animals, such as CF1 mice.

Clearance is evaluated by determining whether the pharmaceutical composition enhances clearance of the infectious agent from the animal. This is typically determined from a sample of biological fluid, such as blood, peritoneal fluid, or cerebrospinal fluid. The infectious agent is cultured from the biological fluid in a manner suitable for growth or identification of the surviving infectious agent. From samples of fluid taken over a period of time after treatment, one skilled in the art can determine the effect of the pharmaceutical composition on the ability of the animal to clear the infectious agent. Further data may be obtained by measuring over a period of time, preferably a period of days, survival of animals to which the pharmaceutical composition is administered. Typically, both sets of data are utilized. Results are considered positive if the pharmaceutical composition enhances clearance or decreases mortality. In situations in which there is enhanced organism clearance, but the test animals still perish, a positive result is still indicated.

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EXAMPLE 1

The Production of Hybridomas and Monoclonal Antibodies

To produce monoclonal antibodies that were directed against the surface proteins of *S. epidermidis* and were opsonic and protective for *S. epidermidis*, mice were immunized with whole *S. epidermidis*, Strain Hay.

A suspension of heat killed *S. epidermidis* was adjusted to an optical density (OD) of 0.137 at a wavelength of 650 nm when measured through a 1 centimeter light path. Bacteria from five mls of the suspension were pelleted by centrifugation (approximately 1800 X g, 10 minutes, room temperature). The supernatant was discarded and the pellet resuspended in 0.6 mls of PBS, which was then mixed with 0.9 mls of complete Freund's adjuvant (CFA). The resulting emulsion was used as the immunogen.

Adult, female BALB/c mice, obtained from Harlan Sprague Dawley (Indianapolis, IN) were immunized subcutaneously with 0.2 mls of the immunogen described above. The mice received a booster immunization approximately two and ½ months later with antigen prepared as described above, except that incomplete Freund's adjuvant (IFA) was used as the adjuvant instead of CFA. A final, prefusion boost was given approximately two months after that. This boost consisted of 1 ml of *S. epidermidis* suspension ($OD_{650}=0.137$). Mice 8159 and 8160 each received an intraperitoneal injection of 0.5 mls of the suspension. Five days later, the spleen from mouse 8159 was removed and used for hybridoma formation.

Hybridomas were prepared by the general methods of Shulman, Wilde and Kohler Nature 276:269-270 (1978) and Barta and Hirshaut "Current Methods in Hybridoma Formation in Methods of Hybridoma Formation, Barta and Heishaut, eds., Humana Press, Clifton, New Jersey (1987). A total of 2.135×10^8 spleenocytes from mouse 8159 were mixed with 2.35×10^7 SP2/0 mouse myeloma cells (ATCC Catalog number CRL1581) and pelleted by centrifugation ($400 \times g$, 10 minutes at room temperature) and washed in serum free medium. The supernatant was removed to near-dryness and fusion of the cell mixture was accomplished in a sterile 50 ml centrifuge conical by the addition of 1 ml of polyethylene glycol (PEG; mw 1400; Boehringer Mannheim) over a period of 60-90 seconds. The PEG was diluted by slow addition of serum-free medium in successive volumes of 1, 2, 4, 8, 16 and 19 mls. The hybridoma cell suspension was gently resuspended into the medium and the cells pelleted by centrifugation ($500 \times g$, 10 minutes at room temperature). The supernatant was removed and the cells resuspended in medium RPMI 1640, supplemented with 10% heat-inactivated fetal bovine serum, 0.05 mM hypoxanthine and 16 uM thymidine (HT medium). One hundred ul of the hybridoma cells were planted into 760 wells of 96-well tissue culture plates. Eight wells (column 1 of plate A) received approximately 2.5×10^4 SP2/0 cells in 100 ul. The SP2/0 cells served as a control for killing by the selection medium added 24 hours later.

Twenty four hours after preparation of the hybridomas, 100 ul of RPMI 1640, supplemented with 10% heat-inactivated fetal bovine serums, 0.1 mM hypoxanthine, 0.8 uM aminopterin and 32 uM thymidine (HAT medium) was added to each well.

Ninety six hours after the preparation of the hybridomas, the SP2/0 cells in plate A, column 1 appeared to be dead, indicating that the HAT selection medium had successfully killed the unfused SP2/0 cells.

Eleven days after the preparation of the hybridomas, supernatants from all wells were tested by ELISA for the presence of antibodies reactive with methanol-fixed *S. epidermidis*. Based on the results of this preliminary assay, cells from 20 wells were transferred to a 24-well culture dish. Four days later, supernatant from these cultures were retested by ELISA for the presence of antibodies reactive with methanol-fixed *S. epidermidis*. Of these supernatants, one (from colony 96-105CE11) was a strongly reactive IgG (Table 1). Two colonies (96-105FD4 and 96-105GB5) were very weakly reactive IgG's and one colony 96-105HB10 was a weakly reactive IgM. Antibodies of the IgM isotype are not as desirable as IgG's and culture 96-105HB10 was cryopreserved and not further examined.

Cultures 96-105 CE11, FD4 and GB5 were reanalyzed several days later and only CE11 showed a strong response (Table 2). No response was obtained with the other cell cultures, and no further experimental work was done with them.

To further test the specificity of this antibody for *S. epidermidis*, a whole cell ELISA with several bacteria was performed (Table 3). The antibodies from this colony bound strongly to *S. epidermidis* (Hay) O.D. 1.090 and to a lesser degree to Group B streptococcus (GBS), but not to *H. influenzae* (HIB+, with type b capsule; HIB- without typable capsule) or type 14, pneumococcus (Pn 14).

A clone from 96-105CE11 IF6 was isolated and retested and was an IgG-1 that reacted strongly with *S. epidermidis* (Strain Hay) in the whole cell ELISA (Table 4). This clone was then designated 96-110. To determine if 96-110 had the broad binding characteristics we sought and would be consistent with binding to the surface protein on *S. epidermidis* (Strain Hay) that bound broadly opsonic antibody, we ran a whole cell ELISA against several coagulase negative staphylococci (Table 5). Using 96-110 in Ascites fluid, strong binding at several dilutions was detected for *S. epidermidis* type I, II, III, *S. hemolyticus* and *S. hominus*.

In addition, 96-110 MAB was purified over a protein G column (Pharmacia). Using a modification of the whole cell ELISA, peroxidase labeled protein A was mixed with the purified 96-110 MAB and then reacted with *S. aureus* type 5 (SA5) and *S. aureus* type 8 (SA8) obtained from ATCC at Accession Nos. 12602 and 12605, respectively. Both *S. aureus* serotypes reacted strongly with the 96-110 MAB (Table 6). Since, in our previous studies, we found that absorption with *S. epidermidis* (Strain Hay) could decrease IgG opsonic activity and opsonic antibodies raised against Hay reacted with a surface protein of Hay, we felt that this was still consistent with a MAB to the surface protein we were trying to characterize. This finding was also important since types 5 & 8, *S. aureus* are serotypes commonly associated with human infections. Using this protein A assay, MAB to type 14 pneumococcus did not demonstrate binding to *S. aureus*.

Therefore, we have identified a mouse IgG₁ MAB raised against *S. epidermidis* (Strain Hay) that binds to the surface of both coagulase negative and coagulase

positive Staphylococci of Gram positive bacteria. Such an antibody would be valuable to prevent or treat infections of Gram positive cocci by preventing attachment of bacteria to epithelial cells or foreign bodies, by enhancing opsonization and protection from infection and by reducing (down modulating) the inflammatory response.

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TABLE 1

Immunoassay Results, 96-105 Supernatants on
Methanol-Fixed S. Hay

| Colony ID | Detection Specific For: | | |
|--------------|-------------------------|-------|-------|
| | G | A | M |
| PBS-F | 0.070 | 0.080 | 0.050 |
| CE11 | 0.788 | 0.065 | 0.056 |
| EB5 | 0.079 | 0.065 | 0.053 |
| EE5 | 0.084 | 0.069 | 0.055 |
| FD4 | 0.089 | 0.067 | 0.059 |
| FG4 | 0.087 | 0.065 | 0.065 |
| FG8 | 0.090 | 0.060 | 0.062 |
| FF9 | 0.095 | 0.062 | 0.059 |
| GE4 | 0.074 | 0.067 | 0.059 |
| GB5 | 0.155 | 0.077 | 0.078 |
| GB6 | 0.073 | 0.062 | 0.053 |
| GC6 | 0.069 | 0.062 | 0.052 |
| GC9 | 0.076 | 0.062 | 0.053 |
| GB10 | 0.075 | 0.064 | 0.102 |
| HG2 | 0.195 | 0.067 | 0.059 |
| HG3 | 0.079 | 0.066 | 0.060 |
| HE4 | 0.076 | 0.073 | 0.065 |
| HG4 | 0.077 | 0.101 | 0.061 |
| HG5 | 0.077 | 0.062 | 0.058 |
| HC8 | 0.083 | 0.064 | 0.057 |
| HB10 | 0.070 | 0.064 | 0.223 |
| AC4 IID10* | 0.065 | 0.066 | 0.069 |

*Monoclonal antibody reactive with Hib protein D.

TABLE 2

Immunoassay Results, 96-105 Supernatants on
Methanol-Fixed S. Hay

| Colony ID | Detection Specific For: | | |
|--------------|-------------------------|-------|-------|
| | G | A | M |
| Buffer | 0.052 | 0.045 | 0.045 |
| CE11 | 0.933 | 0.049 | 0.046 |
| FD4 | 0.073 | 0.054 | 0.051 |
| GB5 | 0.050 | 0.040 | 0.036 |

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TABLE 3

Immunoassay Results, 96-105 Supernatants on
Methanol-Fixed Bacteria

| Colony ID | Detection Antibody | Hay | Hib+ | Hib- | GBS | Pn14 |
|--------------|-----------------------|-------|-------|-------|-------|-------|
| CE11 | gamma-specific | 1.090 | 0.106 | 0.068 | 0.304 | 0.063 |
| FE11 | gamma-specific | 0.167 | 0.084 | 0.068 | 0.112 | 0.053 |
| Buffer | gamma-specific | 0.048 | 0.075 | 0.056 | 0.070 | 0.053 |

Several colonies from 96-105 not cloned.

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TABLE 4

Assay of 96-105 CE11 IF6 on Various Bacteria

| Antibody | Antigen | Dilution | Isotype | Hay | Pn14 |
|----------------|---------|----------|----------|-------|-------|
| PBS-T | | | | 0.072 | 0.064 |
| 96-105CE11-IF6 | Hay | 2 | IgG-1, k | 1.608 | 0.099 |
| | | 4 | | 1.184 | 0.087 |
| | | 8 | | 0.846 | 0.069 |
| | | 16 | | 0.466 | 0.074 |

T06290"5T9E6B60

TABLE 5

Detection of Bacteria of Anti-Hay Monoclonal*
in Whole Cell ELISA

| Antibody | Dilution | Staph. Hay | Staph. Epi I | Staph. Epi II | Staph. Epi III | Staph. Hemmolyt. | Staph. Hominus |
|-------------------|----------|---------------|-----------------|------------------|-------------------|---------------------|-------------------|
| Buffer | | 0.056 | 0.063 | 0.066 | 0.055 | 0.058 | 0.074 |
| 96-110 Ascites | 100 | 1.448 | 2.334 | 1.524 | 1.241 | 1.197 | 0.868 |
| | 400 | 1.325 | 2.542 | 0.746 | 0.425 | 0.830 | 0.422 |
| | 1600 | 1.087 | 2.452 | 0.369 | 0.176 | 0.680 | 0.185 |
| | 6400 | 0.930 | 2.430 | 0.195 | 0.089 | 0.602 | 0.110 |
| | 25600 | 0.674 | 1.672 | 0.113 | 0.069 | 0.647 | 0.081 |

*Anti-Hay Monoclonal from unpurified ascites fluid

TABLE 6

Detection of Methanol-Fixed SA5, SA8 and S. Hay
By Purified Monoclonal Anti-Hay Using Protein A

| Anti-Hay Dilution | ATCC SA5 | ATCC SA8 | USU Hay |
|----------------------|-------------|-------------|------------|
| 500 | 1.329 | 3.345 | 3.017 |
| 1000 | 1.275 | 2.141 | 2.266 |
| 2000 | 0.873 | 1.016 | 1.487 |
| 4000 | 0.333 | 0.491 | 0.951 |
| 8000 | 0.159 | 0.232 | 0.490 |
| 16000 | 0.132 | 0.149 | 0.331 |
| Normal Mouse 1000 | 0.101 | 0.090 | 0.082 |
| Buffer | 0.102 | 0.113 | 0.152 |

Purified anti-Hay stock = 1.63 mg/ml

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EXAMPLE 2

The Opsonic Activity of the Monoclonal Antibody

Antibodies which bind to an antigen may not necessarily enhance opsonization or enhance protection from infection. Therefore, a neutrophil mediated bactericidal assay was used to determine the functional activity of antibody to *S. epidermidis*. Neutrophils were isolated from adult venous blood by dextran sedimentation and ficoll-hypaque density centrifugation. Washed neutrophils were added to round-bottomed wells of microtiter plates (approximately 10^6 cells per well) with approximately 3×10^4 mid-log phase bacteria (*S. epidermidis* Hay, ATCC 55133). Newborn lamb serum (10 uls), screened to assure absence of antibody to *S. epidermidis*, was used as a source of active complement.

Forty microliters of immunoglobulin (or serum) were added at various dilutions and the plates were incubated at 37°C with constant, vigorous shaking. Samples of 10 uls were taken from each well at zero time and after 2 hours of incubation. Each was diluted, vigorously vortexed to disperse the bacteria, and cultured on blood agar plates overnight at 37°C to quantitate the number of viable bacteria. Results are presented as percent reduction in numbers of bacterial colonies observed compared to control samples.

Since the 96-110 MAB bound to both coagulase negative and coagulase positive Staphylococci, opsonic studies were performed to determine if the MAB enhanced phagocytosis and killing of both groups of staphylococci. At a 1:80 dilution, the MAB

enhanced opsonization and killing of coagulase negative Staphylococcus (*S. epidermidis*) to 100%, compared with 49.5% with C' and PMN alone (Table 7). The coagulase positive Staphylococcus also showed enhanced phagocytosis at 1:10 and 1:40 dilution (83.3% and 78.9% respectively) compared with 53.7 percent with C' and PMN alone. At 1:80 dilution, the opsonic activity against *S. aureus* was decreased to 61%.

These data show that not only does the MAB bind to the surface of both coagulase negative and coagulase positive Staphylococci, but that it has functional activity and can enhance phagocytosis and killing of these bacteria. Such an antibody would be capable of promoting clearance of Staphylococci that have invaded a host and would be useful therapeutic agent.

TABLE 7
Opsonic Assay
Antibody: Purified M X Hay, 96-110

| Group Description | Ab Dilution | % Killed <u><i>S. epidermidis</i></u> | % Killed <u><i>S. aureus</i></u> |
|----------------------|----------------|--|-------------------------------------|
| C' only | | 0.0 | 0.0 |
| PMN only | | 0.0 | 0.0 |
| PMN + C' No Ab | | 49.5 | 53.7 |
| PMN + Ab + C' | 10 | - | 83.3 |
| | 40 | - | 78.9 |
| | 80 | 100.0 | 61.0 |

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In vivo Protective Efficacy

Opsonic antibody correlates with enhanced protection from staphylococcal infections, as set forth in the recent series of Fischer applications and issued patent described and incorporated by reference above. To further demonstrate that the MAB can enhance survival to infections with both coagulase positive and coagulase negative Staphylococci, studies were conducted using lethal infection models.

Two day old Wistar rats were injected with $\sim 10^6$ *S. aureus* (type 5, ATCC 12605) subcutaneously just cephalad to the tail. Approximately 30 minutes before and 24 and 48 hours after infection, 0.2 ml MAB 96-110 (~ 320 ug) was given IP. Control animals were given an equal volume of saline or a control MAB not directed against *Staphylococci*. All animals were observed daily for five days to determine survival.

MAB 96-110 enhanced survival in this lethal neonatal model of coagulase positive staphylococcus sepsis (Figure 3): 8/15 survived after treatment with MAB 96-110, and 0/10 survived with Control MAB or 2/25 with saline treatment.

In a similar manner MAB 96-110 enhanced survival in adult mice infected with coagulase negative staphylococci. Adult CF1 mice were given 0.5 ml *S. epidermidis* (Hay) IP (3.5×10^9 bacteria). About 24 hrs and 2 hrs before and 24 hrs post-infection, 320 ug of MAB 96-110 were given to one group of mice and compared with a second group infected in the same manner, but not treated with antibody. All animals were followed 5 days to determine survival. Approximately 23 hours after infection, 70% of

the animals treated with MAB 96-110 were alive compared with 20% of animals not given antibody (Figure 4). When the study was terminated 50% of the MAB animals remained alive compared to only 10% of controls.

Thus, MAB 96-110 could enhance survival in lethal coagulase positive and coagulase negative staphylococcal infections. This enhancement occurred in an adult model and an immunocompromised model (immature neonatal immune system).

EXAMPLE 4

Peptide selection Panning random 6mer and 15mer fd-tet phage libraries

Amplified random 6mer and 15mer fd-tet phage libraries were panned against the 96-110 antibody to yield populations of 6 and 15 amino acid length peptides which cross react with the 96-110 antibody. The original libraries were acquired from George P. Smith, Division of Biological Sciences, University of Missouri, Columbia, MO. In order to be used for panning, the 96-110 antibody was crosslinked to Biotin using the Sulfo-NHS-biotin ester crosslinking kit following the manufacturers protocol (Pierce Chemical Co.).

For the first round of panning, 35mm polystyrene petri dishes (Costar) were coated with streptavidin by incubating the plates overnight at 4°C rocking with 100mM NaHCO₃ and 10ug streptavidin. Streptavidin was then discarded and plates were filled with blocking solution (0.1M NaHCO₃, 5mg/ml dialyzed BSA, 0.1ug/ml streptavidin) and incubated for 1hr at 4°C. The following protocol was then followed: Wash dishes six

min at 10,000rpm, discard supernatant and redissolve phage pellet in 1ml TBS. Collect resuspended phage into a 1.5ml eppendorf tube, clarify the suspension by centrifugation, and add 150ul PEG/NaCl. Allow to incubate on ice for 1 hr. Microfuge the tube 10min, discard supernatant, and redissolve phage in 200ul TBS.

The second and third round of panning are carried out the same way. The eluted, amplified phage (100ul) from the previous panning is preincubated with biotinylated 96-110 antibody (100nM for the second round; 0.1nM for the third round) overnight at 4°C. 400 ul TBS/Tween is added to the mixture and it is pipetted onto streptavidin coated plates (prepared as previously stated) and then incubated with rocking gentle at room temperature for 10 min. The plates are then washed, eluted, and amplified as previously stated. The input and resultant phage are titered to determine yield from biopanning.

EXAMPLE 5

Sequencing resulting phage populations to identify consensus sequences

After the third round of panning, the titered infection plates are used to pick 20 single isolated colonies for each library. The colonies are grown overnight in 5ml NZY medium containing 40ug/ml tetracycline. Replicative form DNA is extracted from each culture using Qiaplasid quick prep kit (Qiagen Inc.) following the manufacturer's protocol. Media supernatants are saved for phage stock to be used in Example 4. 2.5ul of each RF DNA sample is added to a reaction containing 3.5pmole CLC502

primer (5'-TGAATTTTCTGTATGAGGTTT-3') (SEQ ID NO 3), 8 UI Prizm sequencing mix (ABI Inc.), QS to 20ul with water and amplified following manufacturers protocol. Successful sequences are translated and aligned. 18 resulting sequences for the 6mer library panning are listed in Figure 5. 18 resulting sequences for the second experiment 15mer library panning are listed in Figure 6. 17 resulting sequences for the first experiment 15mer library panning are listed in Figure 7. A master list was compiled of the common resulting peptide sequences from all the pannings (Figure 8) with the frequency of occurrence listed to the right of each sequence. Consensus portions of the sequences are marked on the master list (Figure 8).

EXAMPLE 6

Phage EIA comparing 3rd round phage isolates crossreactivity to 96-110 antibody

The saved media phage stocks for each of the common resulting peptide sequences were amplified as previously stated. Amplified phage preparations were quantitated by Abs₂₆₉ and diluted to 1×10^{13} vir/ml and serially diluted 1:2 seven times. A 96-well polystyrene plate was coated with 2ug/ml streptavidin in 0.1M NaHCO₃ overnight at 4°C. Plates were emptied and blocked for 1hr at room temperature with phage blocking solution, 100ul/well. The following protocol was then followed. Wash wells three times with TBS/Tween. Incubate overnight at 4°C with 0.2ug/ml biotinylated 96110 in phage blocking solution, 100ul/well. Wash wells three times with TBS/Tween. Incubate overnight at 4°C with serially diluted phage, 100ul/well. Wash wells three

times with TBS/Tween. Incubate 1 hr at room temperature with 1:5000 goat polyclonal anti-phage-HRP. Wash wells three times with TBS/Tween. Develop with 100ul ABTS substrate (Kirkegaard Perry) for 10-15min and read absorbance (402nm) on spectrophotometer according to manufacturer's protocol. Optical density signals of each phage isolate at 6.25×10^{11} vir/ml are compared in Figure 9. The two isolates yielding the greatest signals are:

15mer 2.12 W R M Y F S H R H A H L R S P (SEQ ID NO:1)

15mer 2.1 W H W R H R I P L Q L A A G R (SEQ ID NO:2)

EXAMPLE 7

Antibodies Against Lipoteichoic Acid (LTA)

As set forth above, we identified two peptides that reacted with 96-110 MAB. However, after identifying the peptides, the sequences did not correspond to any known proteins. Thus we began to consider other possible antigen candidates. We were surprised to find that MAB 96-110 bound strongly to LTA from several gram positive bacteria such as *S. mutans*, *S. aureus* and *S. faecalis* (Table 8). In addition, in an ELISA, when the wells coated with *S. epidermidis* (Strain Hay) were reacted with MAB 96-110 inhibited by varying concentrations of LTA (from *S. mutans*, *S. aureus* and *S. faecalis*), reduction in MAB binding occurred (Table 9). The inhibition of MAB 96-110 binding was greatest at the highest concentration of LTA inhibitor (9 ug/ml for each LTA) and varied according to which bacterial LTA was used (52% inhibition with *S. mutans*, 40.6% with *S. aureus* and 38.2% with *S. faecalis*).

The MAB 96-110 was also analyzed for binding to LTA from *S. pyogenes* (group A streptococcus) and various group A streptococcal M types. The MAB showed strong binding to the LTA and also bound to the different M types with strongest binding to M1 and M3 (Table 10).

We were surprised to find an antibody that bound to LTA and enhanced opsonization for both coagulase positive and coagulase negative staphylococci *in vitro* and enhanced survival in lethal models of staphylococcal (coagulase negative and coagulase positive) sepsis, *in vivo*. This is particularly surprising because the bacteria in each model were injected systemically (SQ or IP) and by-passed the epithelial barriers (skin or mucous membranes) where LTA is thought to possibly act as an adherence factor for the bacteria to epithelial cells.

In addition, this strong anti-LTA reactivity will provide a method to block the binding of LTA bearing bacteria to epithelial cells and prevent colonization of important pathogens such as staphylococci, group A streptococci, *S. faecalis* (enterococci) and *S. mutans*. Since LTA induces proinflammatory cytokines such as TNF, IL-6 and Interferon gamma, MABs with strong anti-LTA binding will also have an anti-inflammatory action and modulate cytokine production secondary to LTA bearing bacteria. Anti-LTA antibodies or vaccines could be designed and produced to modulate cytokine production and inflammation in tissues and prevent the adverse effects of these proinflammatory cytokines.

TABLE 8

Reactivity of Anti-Hay MAB 96-110 on wells Coated with Several LTA's

| Antibody ID | Concentration or Dilution | LTA from <i>S. mutans</i> | LTA from <i>S.</i> <i>aureus</i> | LTA from <i>S.</i> - <i>faecalis</i> |
|-------------|------------------------------|------------------------------|-------------------------------------|---|
| Buffer | - | 0.145 | 0.172 | 0.140 |
| Anti-Hay | 0.9 ug/ml | 3.899 | 3.253 | 3.153 |
| MAB | 0.3 ug/ml | 3.523 | 2.824 | 2.769 |
| 96-110 | 0.1 ug/ml | 2.023 | 2.421 | 2.133 |
| | 0.033 ug/ml | 2.143 | 1.590 | 1.539 |
| | 0.011 ug/ml | 1.396 | 0.998 | 0.832 |

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TABLE 9

Inhibition of Anti-Hay MAB 96-110
with LTA From Different Gram Positive Bacteria

| LTA Inhibitor (ug/ml) | LTA <i>S. mutans</i> | LTA <i>S. aureus</i> | LTA <i>S. faecalis</i> |
|--------------------------|-------------------------|-------------------------|---------------------------|
| 9 | 0.298 | 0.360 | 0.140 |
| 3 | 0.449 | 0.434 | 0.496 |
| 1 | 0.549 | 0.538 | 0.545 |
| 0.37 | 0.558 | 0.526 | 0.549 |
| 0.12 | 0.509 | 0.735 | 0.582 |
| 0.04 | 0.574 | 0.614 | 0.671 |
| 0 | 0.621 | 0.607 | 0.648 |

NOTES:

1. Wells were coated with methanol-fixed Hay.
2. Wells were blocked with 1% BSA in PBS.
3. Monoclonal anti-Hay was used at a final concentration of 0.5 ug/ml and reacted with inhibitors at the concentrations indicated in the Table.
4. Detection was with a gamma-specific Rabbit anti-Mouse.
5. Substrate was TMB.

TABLE 10

Reactivity of MAB 96-110 on Whole Methanol Fixed Group A Strep

| Antibody ID | Dilution or Conc. | GAS* Type 1 #12344 | GAS Type 3 #21546 | GAS Type 18 #12357 | GAS Type 24 #10782 | Response on pyogenes LTA |
|--------------|-------------------|--------------------------|-------------------------|--------------------------|--------------------------|-----------------------------|
| Buffer | | 0.511 | 0.161 | 0.234 | 0.148 | 0.075 |
| Anti-Hay MAb | 0.3 ug/ml | 1.377 | 1.113 | 0.844 | 0.566 | -- |
| Anti-Hay Mab | 0.1 ug/ml | 1.016 | 0.553 | 0.555 | 0.402 | 2.228 |

*All Group A Streptococcus (GAS) from ATCC (accession numbers noted above); plates were coated with MeOH-fixed bacteria and read at 15 minutes.

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EXAMPLE 8

Humanization of the Anti-Staph antibody 96-110 Cloning of the 96-110 variable region cDNAs

The hybridoma cell producing the 96-110 antibody was obtained as described above. A vial of cells was thawed, washed with serum free medium and then resuspended in IMDM (Mediatech) complete media supplemented with 10%FBS (Irvine). Total RNA was isolated from 1×10^8 96-110 cells using the Midi RNA Isolation kit (Qiagen) following the manufacturer's procedure. The RNA was dissolved in 10mM Tris, 0.1mM EDTA (pH8.4) containing 0.03U/ μ g Prime RNase Inhibitor (5'-3') to a final concentration of 0.25 μ g/ μ l.

Figure 10 shows the strategy for cloning the variable region gene fragments and Figure 11 lists the oligonucleotides primers used. The 96-110 total RNA (2 μ g) was converted to cDNA by using Superscript II-MMLV Reverse transcriptase (Life Technologies) and mouse kappa (OKA57) and mouse heavy chain (JS160-162)-specific priming according of manufacturer's procedures. The first strand cDNA synthesis products were then purified using a Centricon-30 concentrator device (Amicon). Of the 40 μ l of cDNA recovered, 5 μ l was used as template DNA for PCR. Typical PCR amplification reactions (100 μ l) contained template DNA, 50 pmoles of the appropriate primers (PMC12-15,55 and OKA57 for tight chains, JSS1-4,8 and JS 160-162 for heavy chains), 2.5 units of *ExTaq* polymerase (PanVera), 1x *ExTaq* reaction buffer, 200 μ M dNTP, 1mM $MgCl_2$. The template was denatured by an initial incubation at 96°C for 5 min. The products were amplified by 15 thermal cycles of

55°C for 30 sec., 70°C for 30 sec, then 96°C for 1 min. followed by 25 step cycles of 70°C for 1 min., then 96°C for 1 min. The PCR products from the successful reactions were purified using the Wizard PCR Purification system (Promega) as per manufacturer's procedure.

The heavy chain PCR products (approximately 400 bp) were then cloned into a bacterial vector for DNA sequence determination. Ligations of the PCR fragments were carried out into the PCR2.1 (invitrogen) T/A style cloning vector following the manufacturer's procedures using a 3:1 insert to vector molar ratio. One half (5 ul) of the ligation reactions were used to transform Ultracompetent XL2Blue cells (Stratagene) as per the manufacturer's procedure. Plasmid clones containing DNA inserts were identified using diagnostic restriction enzyme digestions with NcoI (New England Biolabs). The DNA sequence of plasmids (pJRS308) containing inserts of the appropriate size (400bp) was then determined. The final consensus DNA sequence of the heavy chain variable regions is shown in Figure 12.

The light chain PCR products were treated differently. The hybridoma cell line that expresses the 96-110 antibody was made by fusing mouse spleenocytes with the SP20 myeloma cell line. The SP20 cell line transcribes a pseudogene for the kappa light chain. In addition, the hybridoma cell line that expresses the 96-110 antibody transcribes a second pseudogene product for a kappa light chain that apparently arose from the spleenocyte partner of the hybridoma fusion event. This second pseudogene transcript can be expressed from an expression vector transfected into mammalian cells, but this recombinant antibody product does not bind to heat-killed Staph HAY

kappa exon so that after post transcriptional splicing a mouse human chimeric kappa light chain is produced.

The backbone of the vector was the plasmid pCDNA3 (Invitrogen). This plasmid was cut with HindIII/XhoI and a "light chain polylinker" DNA fragment was inserted to create the stated "light chain vector." This linker contained the restriction sites HindIII, KpnI, ClaI, PmlI, EcoRV XmaI, BamHI and XhoI to facilitate subsequent cloning steps to create the plasmid pCDNA3.LCPL. A SmaI/ BclI DNA fragment containing a light chain leader, anti-CKMB kappa light chain genomic fragment, and 3' UTR was cloned into the EcoRV/BamHI sites of pCDNA3.LCPL. The mouse kappa intron, exon and 3' UTR in this fragment was derived from LCPXK2 received from Dr. Richard Near (Near, RI et al, 1990, Mol Immunol. 27:901-909). Mutagenesis was then performed to eliminate an NruI (209), MluI (229). and BstBI (2962) and to introduce an NheI (1229) and a BamHI (1214) site to create pCDNA3mut.LCPL.LCVK.

A second "heavy chain vector" was constructed from the pCDNA3mut.LCPL.LCVK plasmid by replacing the light chain expression region (HindIII/XhoI) with a "heavy chain polylinker" consisting of restriction sites HpaI, BspEI, EcoRV, KpnI, and XhoI. A SmaI/ KpnI DNA fragment contains a heavy chain leader, antiCKMB IgG2b chain genomic fragment. A KpnI/SalI oligo nucleotide fragment containing a 3' UTR and a NotI upstream of the SalI site was subsequently cloned into the KpnI1XhoI digested plasmid, (knocking out the XhoI site) to create the plasmid pCDNA3mut.HCPL.HCV2b. From this point two vectors were created that did not have any of the anti-CKMB variable or constant domain DNA sequences. This was done by cutting the plasmid

pCDNA3mut.LCPL.LCVK with EcoRV/XhoI and inserting a linker oligonucleotide fragment containing EcoRV, BstBI, and XhoI sites to create pSUN9. In a similar way, the anti-CKMB fragment in pCDNA3mut.HCPL.HCV2b (NruI/NotI) was replaced by a linker oligonucleotide fragment containing NruI, EcoRI and NotI sites to create pSUN10. A human kappa light chain constant domain was then cloned into pSUN9 as a BstBI/XhoI fragment, and a human IgG1 constant domain was cloned into pSUN10 as a EcoRI/NotI fragment. A BglII/NheI fragment from the human heavy chain vector was then cloned into the human light chain vector cut with BamHI/NheI to create pSUN15. This vector results in the production of recombinant antibody molecules under the control of the CMV transcriptional promoters. The heavy chain molecules are direct cDNA constructs that fuse the variable region sequence directly into the human IgG1 constant domain. The light chain molecules, on the other hand, have a mouse kappa intron region 3' of the variable region coding fragment. After splicing the variable region becomes fused to a human kappa constant region exon. The selectable marker for the vector in mammalian cells is Neomycin (G418).

The variable region gene fragments were re-amplified by PCR using primers that adapted the fragments for cloning into the expression vector (see Figures 12 and 14). The heavy chain front primer (96110HF2) includes a 5' tail that encodes the C-terminus of the heavy chain leader and an *NruI* restriction site for cloning, while the heavy chain reverse primer (96110HB) adds a 3' *EcoRI* restriction site for cloning. The light chain front primer (96110bLF) converts the first amino acid of the 96-110 light chain variable region sequence from glutamine (Q) to aspartic acid (D) via the introduction of an

EcoRV restriction site at the N-terminus of the light chain variable region for cloning, while the light chain reverse primer (96-110bLB) adds a 3' DNA sequence for the joining region-kappa exon splice junction followed by a *BstB1* restriction site for cloning. Because the last amino acid of the light chain variable region is an arginine (R) which is a very rare amino acid at this position, the reverse primer introduces a point mutation in the codon for amino acid 106 that converts it to the much more common lysine (L). This was done because the splice junction in the expression vector for the kappa chain was derived from a J region that encoded a lysine at this position. Neither mutation in the recombinant form of the antibody would be anticipated to alter the antibodies binding characteristics.

PCRs were performed as described above except 10ng of plasmid template was used in each case. Following a 5 min. incubation at 96°C, the PCR parameters were 35 thermal cycles of 58°C for 30 sec., 70°C for 30 sec., and 96°C for 1 min. The 96-110 heavy chain PCR product (approximately 400 bp) was digested with *NruI* and *EcoRI* (New England Biolabs), purified using a Qiaquick PCR Purification column (Qiagen), as described by the manufacturer, and ligated into *NruI* / *EcoRI* digested and gel-purified pSUN15, resulting in plasmid pJRS311 (see Figure 13).

At this point a *BstBI/NotI* (New England Biolabs) DNA fragment containing a mouse kappa J-kappa intron fragment fused to a human kappa exon fragment was digested and gel-purified from the vector tKMC180C1. This fragment was ligated into the backbone of pJRS311 digested with *BstBI/NotI* and gel-purified resulting in the plasmid pJRS315 (see Figure 13).

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This was the plasmid into which was cloned the 96-110 light chain variable region. The 96-110 light chain PCR product (approximately 350 bp) was digested with *EcoRV* and *BstBI* (New England Biolabs), purified using a Qiaquick PCR Purification column (Qiagen), as described by the manufacturer, and ligated into *EcoRV/Bs/BI* digested and gel-purified pJRS315, resulting in plasmid pJRS326 (see Figure 13).

It was determined that during this cloning process, a deletion of approximately 200bp occurred at the intron exon junction of the kappa light chain. To repair this, an identical DNA fragment (also a *BstBI/NotI* restriction fragment) was gel-purified from digested pEN22 and ligated into *BstBI/NotI* digested and gel-purified pJRS326, resulting in the final expression plasmid construct pJRS334 (see Figures 13 and 14). The sequence of the variable regions and leader and other junctions was verified prior to mammalian cell transfection.

EXAMPLE 9

Transient production of recombinant chimeric mouse/human 96-110 antibody

Two individual clones of the plasmid pJRS334 (pJRS334-1, -2) were transfected into COS and CHO cells using Superfectant (Qiagen) in 6 well tissue culture cells as described by the manufacturer. After three days the supernatant was assayed for the production of "humanized" antibody and for the capability for the expressed antibody to bind to the heat-killed Staph antigen.

Antibody production assays were preformed in 8-well strips from 96-well microtiter plates (Maxisorp F8; Nunc, Inc.) coated at a 1:500 dilution with Goat anti-Human IgG antibody (Pierce) using a bicarbonate coating buffer, pH 8.5. The plates are covered with pressure sensitive film (Falcon, Becton Dickinson) and incubated overnight at 4°C. Plates are then washed once with Wash solution (Imadazole/NaCl/0.4% Tween-20). 100 microliters of culture supernatant was then applied to duplicate wells and allowed to incubate for 30 minutes on plate rotator at room temperatures. The plates were washed five times with Wash solution. A Goat anti Human kappa-HRP (Southern Biotechnologies) conjugate was diluted 1:800 in the sample/conjugate diluent. 100 microliters was added to the samples, then incubated on a plate rotator for 30 minutes at room temperature. The samples were washed as above and then incubated with 100 µL/well of ABTS developing substrate (Kirkgaard & Perry Laboratories) for 10-15 minutes on a plate rotator at room temperature. The reaction was stopped with 100 µL/well of Quench buffer (Kirkgaard & Perry Laboratories) and the absorbance value at 405 nm was determined using an automated microtiter plate ELISA reader (Ceres UV900HI, Biotek, Winooski, Vermont). As a positive control, a humanized mouse/human chimeric antibody BC24 was used. This assay (see Figure 15) demonstrates that the transfection of cells with this plasmid construct to results in the cells producing a molecule containing both human IgG and kappa domains. The supernatants were then assayed for the ability of the expressed antibodies to bind to heat-killed Staph. The activity assays were preformed in 8-well strips from 96-well microtiter plates (Maxisorp F8; Nunc, Inc.) coated at 0.09 OD/well

with heat-killed Staph Hay cell material suspended in MeOH. The plates are left uncovered and incubated overnight at 4°C. Plates are then washed once PBS. 100 microliters of culture supernatant was then applied to duplicate wells and allowed to incubate for 60 minutes on plate rotator at room temperature. The plates were washed five times with Wash solution. The goat anti Human kappa-HRP was diluted 1:800 in the sample/conjugate diluent. 100 microliters was added to the samples, then incubated on a plate rotator for 30 minutes at room temperatures. The samples were washed as above and then incubated with 100 µL/well of ABTS developing substrate (Kirkgaard & Perry Laboratories) for 10-15 minutes on a plate rotator at room temperature. The reaction was stopped with 100 µL/well of Quench buffer (Kirkgaard & Perry Laboratories) and the absorbance value at 405 nm was determined using an automated microtiter plate ELISA reader (Ceres UV900HI, Bioteck, Winooski, Vermont). As a positive control, the original mouse monoclonal antibody 96-110 was used, and assayed with a Goat anti-Mouse Fc-HRP conjugate @ 1:2000 dilution. This assay (see Figure 16) demonstrates that the transfection of cells with this plasmid construct to results in the cells producing a molecule that binds to the Staph Hay cellular antigen.

EXAMPLE 10

Stable production of recombinant chimeric mouse/human 96-110 antibody

The plasmid pJRS334-1 was transfected into NS/O cells (obtainable from Baxter International) and CHO cells using electroporation. The plasmid was linearized with

PvuI restriction digestion. 25 micrograms of digested plasmid DNA was mixed with 1×10^7 cells in a total volume of 800 microliters in a 4 centimeter cuvette and subjected to a pulse of 250mA, 9600microF. The cells were plated out after 24 hours in 10ml non-selective media. The cells were then diluted out into 96-well microtiter plates. As colonies appeared, the supernatants were assayed for the production of "humanized" antibody and for the capability for the expressed antibody to bind to the heat-killed Staph antigen. Antibody production and activity assays for the stable transfectants were performed as described above. These assays demonstrate that the transfection of cells with this plasmid construct can result in the production of a stable cell line that produces a humanized chimeric version of the 96-110 mouse hybridoma antibody.

EXAMPLE 11

Opsonic Activity

Having produced a chimeric anti-LTA MAB for staphylococci, we tested its functional activity using *S. epidermidis* as a representative staphylococcal organism. Using the neutrophil mediated opsonophagocytic assay described generally in the Material and Methods section, we assessed the MAB's opsonic activity by evaluating the percent of bacteria killed after two hours of incubation.

Neutrophils, specifically polymorphonuclear neutrophils, were isolated from adult venous blood by dextran sedimentation and ficoll-hypaque density centrifugation. Washed neutrophils were added to round-bottomed wells of microtiter plates (approximately 10^6 cells per well) with approximately 3×10^4 mid-log phase bacteria (*S.*

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epidermidis Hay, ATCC 55133). Human sera (10 uls), screened to assure absence of antibody to *S. epidermidis*, was used as a source of active complement (C-Barb-Ex (1:4)).

Forty microliters of immunoglobulin were added at various concentrations (20 ug/ml, 40 ug/ml, 80 ug/ml, and 160 ug/ml) and the plates were incubated at 37°C with constant, vigorous shaking. Samples of 10 uls were taken from each well at zero time and after 2 hours of incubation. Each was diluted, vigorously vortexed to disperse the bacteria, and cultured on blood agar plates overnight at 37°C to quantitate the number of viable bacteria. Results are presented in Figure 17 as percent reduction in numbers of bacterial colonies observed compared to control samples.

Compared to PMN alone or PMN plus complement, the addition of the MAB markedly enhanced opsonic activity for staphylococcus at 20-160 ug/ml). These data demonstrate that the MAB has functional activity and can enhance the phagocytosis and killing of staphylococcal organisms, as represented by *S. epidermidis*.

EXAMPLE 12

In vivo Protective Efficacy

Using the lethal staphylococcal sepsis in adult mice assay (described in Example 3), we compared protection between the original mouse MAB and the chimeric HuMAB. Adult CF1 mice were given 0.5 ml *S. epidermidis* (Hay) IP (3.5×10^9 bacteria). About 24 hrs and 1 hr before infection, 14 mg/kg of each MAB was given to a group of mice,

with a third group of mice given only PBS. All animals were followed for 40 hours after challenge to determine survival.

As set forth in Figure 18, approximately 18 hours after infection, all the control animals died while both treatment groups exhibited 100% survival. At 30 hours after infection, both MAB treatment groups exhibited 70% survival. At the end of the study, the group that received the mouse MAB exhibited greater survival than the group receiving the chimeric MAB, but both MAB enhanced survival over the PBS controls.

We conducted further studies with the chimeric MAB at a dose of 18 mg/kg/dose 2 doses given IP 24 and 1 hour prior to infection (3×10^9 IP *S. epidermidis*, Hay). As set forth in Figure 19, the chimeric MAB enhanced survival.

We also assessed the effect of the chimeric MAB on bacteremia in the lethal *S. epidermidis* sepsis model. CF-1 mice were twice infected IP with strain Hay and the chimeric MAB. Bacteremia is expressed as the number of bacteria isolated on blood agar after a 1:1000 dilutions. As set forth in Figure 20, the chimeric MAB reduced bacterial levels by over 2 logs. Additional studies demonstrated that bacteremia was reduced to a greater degree using 40 mg/kg/dose compared to 20 mg/kg/dose even if survival was comparable. See Figure 21.

These data indicate that increasing the amount of antibody resulted in increased bacterial clearance *in vivo*. Such a response is similar to the observed enhanced opsonic activity *in vitro* as seen when antibody was increased from 20 ug/mg to 160 ug/ml in the neutrophil mediated opsonophagocytic assay (Figure 17).

EXAMPLE 13

In vivo Protective Efficacy

The effect of the chimeric MAB 96-110 was then analyzed in a neonatal staphylococcal model using suckling rats with a foreign body infection. Two day old Wistar rats were treated with lipid emulsion (as is standard in newborn care for nutritional purposes) 0.2 ml, 20% IP on day -1 and again on day +1 and +2 to induce further compromise of the immuno system. In two studies, we injected approximately 5×10^7 of four different strains of *S. epidermidis*, identified below in Table 11 SQ through a plastic catheter and the catheter was left in place under the skin. Saline, 0.2 ml, or MAB 96-110, 0.2 ml (dose of 50-60 mg/kg), was given IP 30 min before and 24 hours after infection. The animals were followed for 5 days.

As set forth in Table 11, in study I, survival for animals receiving MAB ranged from 67% to 83%, with an average of 76%, in contrast to saline treatment, which ranged from 33% to 50%, with an average of 39%. Study II showed even more impressive results. Survival for animals treated with MAB ranged from 83% to 100%, with 90% average, compared to the saline controls at 33% to 50%, with an average of 40%. The complied data for study II are shown in Figure 22.

TABLE 11

The Effect of Hu96-110 on Survival in a Lethal Neonatal S. epidermidis Sepsis Model

| Litter Number | <u>S. epidermidis</u> strain | Monoclonal Antibody | | Saline Control | |
|-----------------------|----------------------------------|---------------------|--------------|----------------|--------------|
| | | Treated | Survived (%) | Treated | Survived (%) |
| Study I | | | | | |
| 31 | Haywood (type II) (clinical) | 6 | 4 (67%) | 6 | 2 (33%) |
| 32 | 35984 (type I) (Prototype) | 5 | 4 (80%) | 4 | 2 (50%) |
| 33 | Summer (48357) (clinical) | 6 | 4 (67%) | 6 | 2 (33%) |
| 34 | SE-360 (type III) (Prototype) | 6 | 5 (83%) | 6 | 2 (33%) |
| 35 | Haywood (type II) (clinical) | 6 | 5 (83%) | 6 | 3 (50%) |
| TOTAL | | 29 | 22 (76%) | 28 | 11 (39%) |
| Study II | | | | | |
| 36 | Haywood (type II) (clinical) | 6 | 6 (100%) | 6 | 3 (50%) |
| 37 | 35984 (type I) (Prototype) | 6 | 5 (83%) | 6 | 2 (33%) |
| 38 | Summer (48357) (clinical) | 6 | 6 (100%) | 6 | 2 (33%) |
| 39 | SE-360 (type III) (Prototype) | 6 | 5 (83%) | 6 | 2 (33%) |
| 40 | Haywood (type II) (clinical) | 6 | 5 (83%) | 6 | 3 (50%) |
| TOTAL | | 30 | 27 (90%) | 30 | 12 (40%) |
| TOTAL OF BOTH STUDIES | | 59 | 49 (88%) | 58 | 23 (40%) |

These data demonstrate that the chimeric human antibody directed against LTA is opsonic and enhances survival against staphylococci. In addition, the antibody promotes clearance of the staphylococci from the blood. Thus antibody to LTA provides prophylactic and therapeutic capabilities against staphylococcal infections and vaccines using LTA or peptide mimeotopes of LTA that induce anti-LTA antibodies would also have prophylactic capabilities.

Having now fully described the invention, it will be appreciated by those skilled in the art that the invention can be performed within a range of equivalents and conditions without departing from the spirit and scope of the invention and without undue experimentation. In addition, while the invention has been described in light of certain embodiments and examples, the inventors believe that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the invention which follow the general principles set forth above.

The specification includes recitation to the literature and those literature references are herein specifically incorporated by reference.

The specification and examples are exemplary only with the particulars of the claimed invention set forth as follows:

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(1) GENERAL INFORMATION:

(i) APPLICANT: Fischer, Gerald W.
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Wong, Hing
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(ii) TITLE OF INVENTION: OPSONIC AND PROTECTIVE MONOCLONAL AND CHIMERIC ANTIBODIES SPECIFIC FOR LIPOTEICHOIC ACID OF GRAM POSITIVE BACTERIA

(iii) NUMBER OF SEQUENCES: 105

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US Unassigned

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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trp | Arg | Met | Tyr | Phe | Ser | His | Arg | His | Ala | His | Leu | Arg | Ser | Pro |
| 1 | | | | 5 | | | | | 10 | | | | | 15 |

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

| | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trp | His | Trp | Arg | His | Arg | Ile | Pro | Leu | Gln | Leu | Ala | Ala | Gly | Arg |
| 1 | | | | 5 | | | | 10 | | | | | | 15 |

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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T05390"5T95550

[illegible]

(2) INFORMATION FOR SEQ ID NO:4:

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

74

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGG ANT CAT GCG GAT AGG GTT TAT GGG GCC
Gly Xaa His Ala Asp Arg Val Tyr Gly Ala
1 5 10

30

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Xaa His Ala Asp Arg Val Tyr Gly Ala
1 5 10

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGG GCT TGG CAT TGG CGT CAT CGT ATT CCT CTT CAG CTT GCT GCT GGT 48
Gly Ala Trp His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15
CGT GGG GCC 57
Arg Gly Ala

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly Ala Trp His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15
Arg Gly Ala

00000000000000000000000000000000

(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1..57

[illegible]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Ala Arg Arg His Gly Asn Phe Ser His Phe Phe His Arg Ser Leu
1 5 10 15
Ile Gly Ala

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

| | |
|---|----|
| GGG GCT TGG AAG GCT TTG TTT AGT CAT TCT TAT CGT CCT CGG GGT TCG | 48 |
| Gly Ala Trp Lys Ala Leu Phe Ser His Ser Tyr Arg Pro Arg Gly Ser | |
| 1 5 10 15 | |
| GCT GGG GCC | 57 |
| Ala Gly Ala | |

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

| | |
|---|--|
| Gly Ala Trp Lys Ala Leu Phe Ser His Ser Tyr Arg Pro Arg Gly Ser | |
| 1 5 10 15 | |
| Ala Gly Ala | |

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGG GCT AGG CAT TGG CGT CAT CGT ATT CCT CTT CAG CTT GCT GCT GGT 48
Gly Ala Arg His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15
CGT GGG GCC 57
Arg Gly Ala

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gly Ala Arg His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15
Arg Gly Ala

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGG GCT TGG CAT TGG CGT CAT CGT ATT CCT CTT CAG CTT GCT GCT GGT 48
Gly Ala Trp His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15
CGT GGG GCC 57
Arg Gly Ala

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gly Ala Trp His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15
Arg Gly Ala

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGG GCT TGG CAT TGG CGT CAT CGT ATT CCT CTT CAG CTT GCT GCT GGT 48
Gly Ala Trp His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15

CGT GGG GCC 57
Arg Gly Ala

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gly Ala Trp His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15

Arg Gly Ala

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGG GCT CAG GTG GCT GTT TTG TAT CCT CCT TTG GCT GAT GCT ACT GAG 48
Gly Ala Gln Val Ala Val Leu Tyr Pro Pro Leu Ala Asp Ala Thr Glu
1 5 10 15
CTT GGG GCC 57
Leu Gly Ala

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Ala Gln Val Ala Val Leu Tyr Pro Pro Leu Ala Asp Ala Thr Glu
1 5 10 15
Leu Gly Ala

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGG GCT CGT CGG CAT GGT AAT TTT TCT CAT TTT TTT CAT CGG TCG TTG 48
Gly Ala Arg Arg His Gly Asn Phe Ser His Phe Phe His Arg Ser Leu
1 5 10 15
ATT GGG GCC 57
Ile Gly Ala

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gly Ala Arg Arg His Gly Asn Phe Ser His Phe Phe His Arg Ser Leu
1 5 10 15
Ile Gly Ala

[illegible]

(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1..57

[illegible]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gly Ala Arg Arg His Gly Asn Phe Ser His Phe Phe His Arg Ser Leu
1 5 10 15
Ile Gly Ala

(i) SEQUENCE CHARACTERISTICS:

4

(ix) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCT GGG GCC
Pro Gly Ala

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Pro Gly Ala

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(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1..57

| | | |
|-----|-----|-----|
| G | G | T |
| CCT | GGG | GCC |
| Pro | Gly | Ala |

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gly Ala Trp Arg Met Tyr Phe Ser His Arg His Ala His Leu Arg Ser
1 5 10 15
Pro Gly Ala

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

| | |
|---|----|
| GGG GCT TGG CGG AAG TAT TTT TCT TAT CAT CAT GCG CAT CTT TGT AGT | 48 |
| Gly Ala Trp Arg Lys Tyr Phe Ser Tyr His His Ala His Leu Cys Ser | |
| 1 5 10 15 | |
| CCT GGG GCC | 57 |
| Pro Gly Ala | |

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

| | |
|---|--|
| Gly Ala Trp Arg Lys Tyr Phe Ser Tyr His His Ala His Leu Cys Ser | |
| 1 5 10 15 | |
| Pro Gly Ala | |

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(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1..57

[illegible]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Ala Trp Arg Met Tyr Phe Ser His Arg His Ala His Leu Arg Ser
1 5 10 15
Pro Gly Ala

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGG GCT TGG CGT ATG TAT TTT TCT CAT CGT CAT GCG CAT CTT CGT AGT 48
Gly Ala Trp Arg Met Tyr Phe Ser His Arg His Ala His Leu Arg Ser
 1 5 10 15

CCT GGG GCC 57
Pro Gly Ala

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Ala Trp Arg Met Tyr Phe Ser His Arg His Ala His Leu Arg Ser
 1 5 10 15

Pro Gly Ala

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

| | |
|---|----|
| GGG GCT TGG CGT ATG TAT TTT TCT CAT CGT CAT GCG CAT CTT CGT AGT | 48 |
| Gly Ala Trp Arg Met Tyr Phe Ser His Arg His Ala His Leu Arg Ser | |
| 1 5 10 15 | |
| CCT GGG GCC | 57 |
| Pro Gly Ala | |

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

| | |
|---|--|
| Gly Ala Trp Arg Met Tyr Phe Ser His Arg His Ala His Leu Arg Ser | |
| 1 5 10 15 | |
| Pro Gly Ala | |

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

| | |
|---|----|
| GGG GCT CGT CGG CAT GGT AAT TTT TCT CAT TTT TTT CAT CGG TCG TTG | 48 |
| Gly Ala Arg Arg His Gly Asn Phe Ser His Phe Phe His Arg Ser Leu | |
| 1 5 10 15 | |
| ATT GGG GCC | 57 |
| Ile Gly Ala | |

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

| | |
|---|--|
| Gly Ala Arg Arg His Gly Asn Phe Ser His Phe Phe His Arg Ser Leu | |
| 1 5 10 15 | |
| Ile Gly Ala | |

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GGG GCT TGG CAT TGG CGT CAT CGT ATT CCT CTT CAG CTT GCT GCT GGT 48
Gly Ala Trp His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15
CGT GGG GCC 57
Arg Gly Ala

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Gly Ala Trp His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15
Arg Gly Ala

006230-579550

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GGG GCT CGT CGG CAT GGT AAT TTT TCT CAT TTT TTT CAT CGG TCG TTG 48
Gly Ala Arg Arg His Gly Asn Phe Ser His Phe Phe His Arg Ser Leu
1 5 10 15
ATT GGG GCC 57
Ile Gly Ala

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Ala Arg Arg His Gly Asn Phe Ser His Phe Phe His Arg Ser Leu
1 5 10 15
Ile Gly Ala

000000

(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1..57

GGG GCT GAT TGG ATT ACT TTT CAT CGT CGT CAT CAT GAT CGT GTT CTT 48
Gly Ala Asp Trp Ile Thr Phe His Arg Arg His His Asp Arg Val Leu
1 5 10 15

TCT GGG GCC
Ser Gly Ala

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Gly Ala Asp Trp Ile Thr Phe His Arg Arg His His Asp Arg Val Leu
1 5 10 15

Ser Gly Ala

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GGG GCT GGT TGG ATT ACT TTT CAT CGT CGT CAT CAT GAT CGT GTT CTT 48
Gly Ala Gly Trp Ile Thr Phe His Arg Arg His His Asp Arg Val Leu
1 5 10 15
TCT GGG GCC
Ser Gly Ala 57

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Gly Ala Gly Trp Ile Thr Phe His Arg Arg His His Asp Arg Val Leu
1 5 10 15
Ser Gly Ala

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GGG GCT GGG AAG GCT ATG TTT AGT CAT TCT TAT CGT CAT CGG GGT TCG 48
Gly Ala Gly Lys Ala Met Phe Ser His Ser Tyr Arg His Arg Gly Ser
 1 5 10 15

GCT GGG GCC
Ala Gly Ala 57

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Gly Ala Gly Lys Ala Met Phe Ser His Ser Tyr Arg His Arg Gly Ser
 1 5 10 15

Ala Gly Ala

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GGG GCT GAT TGG ATT ACT TTT CAT CGT CGT CAT CAT GAT CGT GTT CTT 48
Gly Ala Asp Trp Ile Thr Phe His Arg Arg His His Asp Arg Val Leu
1 5 10 15
TCT GGG GCC 57
Ser Gly Ala

(2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Gly Ala Asp Trp Ile Thr Phe His Arg Arg His His Asp Arg Val Leu
1 5 10 15
Ser Gly Ala

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GGG GCT AGT CGT CAT ATG CTT GCT CGG TGG TCG CGT TTG CTT GCT GTT 48
Gly Ala Ser Arg His Met Leu Ala Arg Trp Ser Arg Leu Leu Ala Val
1 5 10 15
CCT GGG GCC 57
Pro Gly Ala

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Gly Ala Ser Arg His Met Leu Ala Arg Trp Ser Arg Leu Leu Ala Val
1 5 10 15
Pro Gly Ala

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GGG GCT GGG AAG GCT ATG TTT AGT CAT TCT TAT CGT CAT CGG GGT TCG 48
Gly Ala Gly Lys Ala Met Phe Ser His Ser Tyr Arg His Arg Gly Ser
1 5 10 15

GCT GGG GCC 57
Ala Gly Ala

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Gly Ala Gly Lys Ala Met Phe Ser His Ser Tyr Arg His Arg Gly Ser
1 5 10 15

Ala Gly Ala

(2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GGG GCT TGG CAT TGG CGT CAT CGT ATT CCT CTT CAG CTT GCT GCT GGT 48
Gly Ala Trp His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15
CGT GGG GCC 57
Arg Gly Ala

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Gly Ala Trp His Trp Arg His Arg Ile Pro Leu Gln Leu Ala Ala Gly
1 5 10 15
Arg Gly Ala

(2) INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GGG GCT CGT CGG CAT GGT AAT TTT TCT CAT TTT TTT CAT CGG TCG TTG 48
Gly Ala Arg Arg His Gly Asn Phe Ser His Phe Phe His Arg Ser Leu
 1 5 10 15

ATT GGG GCC 57
Ile Gly Ala

(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Gly Ala Arg Arg His Gly Asn Phe Ser His Phe Phe His Arg Ser Leu
 1 5 10 15

Ile Gly Ala

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(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..57

[illegible]

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

Gly Ala Trp Lys Ala Leu Phe Ser His Ser Tyr Arg Pro Arg Gly Ser
1 5 10 15
Ala Gly Ala

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GGG GCT CAG GTG GCT GTT TTG TAT CCT CCT TTG GCT GAT GCT ACT GAG 48
Gly Ala Gln Val Ala Val Leu Tyr Pro Pro Leu Ala Asp Ala Thr Glu
1 5 10 15
CTT GGG GCC 57
Leu Gly Ala

(2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Gly Ala Gln Val Ala Val Leu Tyr Pro Pro Leu Ala Asp Ala Thr Glu
1 5 10 15
Leu Gly Ala

(2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGG GCT TGG CGT ATG TAT TTT TCT CAT CGT CAT GCG CAT CTT CGT AGT 48
Gly Ala Trp Arg Met Tyr Phe Ser His Arg His Ala His Leu Arg Ser
1 5 10 15
CCT GGG GCC 57
Pro Gly Ala

(2) INFORMATION FOR SEQ ID NO:65:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Gly Ala Trp Arg Met Tyr Phe Ser His Arg His Ala His Leu Arg Ser
1 5 10 15
Pro Gly Ala

106290"5T66660

[illegible]

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1..30

GGG GCT CAT GCG GAT AGG GTT TAT GGG GCC
Gly Ala His Ala Asp Arg Val Tyr Gly Ala
1 5 10

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Gly Ala His Ala Asp Arg Val Tyr Gly Ala
1 5 10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

ATTTCAGGCC CAGCCGGCCA TGGCCGARGT RMAGCTKSAK GAGWC

45

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

ATTTCAGGCC CAGCCGGCCA TGGCCGARGT YCARCTKCAR CARYC

45

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

ATTTCAGGCC CAGCCGGCCA TGGCCCAGGT GAAGCTKSTS GARTC

45

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

ATTCAGGCC CAGCCGGCCA TGGCCGAVGT GMWGCTKGTG GAGWC

45

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

ATTCAGGCC CAGCCGGCCA TGGCCCAGGT BCARCTKMAR SARTC

45

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

35

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

35

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

```
(ii) MOLECULE TYPE: other nucleic acid
      (A) DESCRIPTION: /desc = "primer"
```

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CCCGGGCCAC CATGGAGACA GACACACTCC TG

32

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

CCCGGGCCAC CATGGATTTT CAAGTGCAGA TTTTC

35

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

CCCGGGCCAC CATGGAGWCA CAKWCTCAGG TC

32

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

TAATATCGCG ACAGCTACAG GTGTCCACTC CCGAAGTGAT GCTGGTGGAG WCTG

54

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

TTATAGAATT CTGAGGAGAC GGTGAGTGAG

30

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

TTAGGCGATA TCGTTCTCTC CAGTCTCC

29

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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GTAACCGTTC GAAAAGTGTA CTTACGTTTT ATTTCCAGCA TGGTCC

(2) INFORMATION FOR SEQ ID NO:86:

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

TCA TTG AAA,CTC TCA TGT GCA GCC TCT GGA TTC ACC TTC AAT 90
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn
20 25 30

(2) INFORMATION FOR SEQ ID NO:87:

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn
20 25 30

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1..57

[illegible]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Asn Tyr Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
1 5 10 15

Общество

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

```
(ix) FEATURE:
      (A) NAME/KEY: CDS
      (B) LOCATION: 1..57
```

[illegible]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

114

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..96

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| AGG | TTC | ACC | ATC | TCC | AGA | GAT | GAT | TCA | CAA | AGC | ATG | CTC | TAT | CTG | CAA | 48 |
| Arg | Phe | Thr | Ile | Ser | Arg | Asp | Asp | Ser | Gln | Ser | Met | Leu | Tyr | Leu | Gln | |
| 1 | | | | 5 | | | | 10 | | | | | | 15 | | |
| | | | | | | | | | | | | | | | | |
| ATG | AAC | AAC | TTG | AAA | ACT | GAG | GAC | ACA | GCC | ATG | TAT | TAC | TGT | GTG | AGA | 96 |
| Met | Asn | Asn | Leu | Lys | Thr | Glu | Asp | Thr | Ala | Met | Tyr | Tyr | Cys | Val | Arg | |
| | | | 20 | | | | | 25 | | | | | 30 | | | |

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Phe | Thr | Ile | Ser | Arg | Asp | Asp | Ser | Gln | Ser | Met | Leu | Tyr | Leu | Gln |
| 1 | | | | 5 | | | | 10 | | | | | | 15 | |
| | | | | | | | | | | | | | | | |
| Met | Asn | Asn | Leu | Lys | Thr | Glu | Asp | Thr | Ala | Met | Tyr | Tyr | Cys | Val | Arg |
| | | | 20 | | | | | 25 | | | | | 30 | | |

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..69

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

CGG GGG GCT TCA GGG ATT GAC TAT GCT ATG GAC TAC TGG GGT CAA GGA 48
Arg Gly Ala Ser Gly Ile Asp Tyr Ala Met Asp Tyr Trp Gly Gln Gly
1 5 10 15
ACC TCA CTC ACC GTC TCC TCA 69
Thr Ser Leu Thr Val Ser Ser
20

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

Arg Gly Ala Ser Gly Ile Asp Tyr Ala Met Asp Tyr Trp Gly Gln Gly
1 5 10 15
Thr Ser Leu Thr Val Ser Ser
20

(2) INFORMATION FOR SEQ ID NO:96:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 69 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..69

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

CAA ATT GTT CTC TCC CAG TCT CCA GCA ATC CTG TCT GCA TCT CCA GGG 48
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15

GAA AAG GTC ACA ATG ACT TGC 69
Glu Lys Val Thr Met Thr Cys
20

(2) INFORMATION FOR SEQ ID NO:97:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys
20

(2) INFORMATION FOR SEQ ID NO:98:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

AGG GCC AGC TCA AGT GTA AAT TAC ATG CAC
Arg Ala Ser Ser Ser Val Asn Tyr Met His
1 5 10

30

(2) INFORMATION FOR SEQ ID NO:99:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

Arg Ala Ser Ser Ser Val Asn Tyr Met His
1 5 10

0593615-062901

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 66 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..66

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

| | |
|---|----|
| TGG TAC CAG CAG AAG CCA GGA TCC TCC CCC AAA CCC TGG ATT TCT GCC | 48 |
| Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Ser Ala | |
| 1 5 10 15 | |
| ACA TCC AAC CTG GCT TCT | 66 |
| Thr Ser Asn Leu Ala Ser | |
| 20 | |

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

| | |
|---|--|
| Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Ser Ala | |
| 1 5 10 15 | |
| Thr Ser Asn Leu Ala Ser | |
| 20 | |

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..96

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

| | |
|---|----|
| GGA GTC CCT GCT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT | 48 |
| Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser | |
| 1 5 10 15 | |
| CTC ACA ATC AGC AGA GTG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC | 96 |
| Leu Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys | |
| 20 25 30 | |

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

| | |
|---|--|
| Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser | |
| 1 5 10 15 | |
| Leu Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys | |
| 20 25 30 | |

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| CAG | CAG | TGG | AGT | AGT | AAC | CCA | CCC | ACG | TTC | GGA | GGG | GGG | ACC | ATG | CTG | 48 |
| Gln | Gln | Trp | Ser | Ser | Asn | Pro | Pro | Thr | Phe | Gly | Gly | Gly | Thr | Met | Leu | |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|--|--|--|--|--|--|--|--|--|--|--|--|--|----|
| GAA | ATA | AGA | | | | | | | | | | | | | | 57 |
| Glu | Ile | Arg | | | | | | | | | | | | | | |

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Gln | Trp | Ser | Ser | Asn | Pro | Pro | Thr | Phe | Gly | Gly | Gly | Thr | Met | Leu |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |

Glu Ile Arg